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FACTORS AFFECTING TESTS FOR VITAMIN A DEFICIENCY

By

Marian K. Lipkind.

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Factors Affecting Tests For Vitamin A Deficiency

A Thesis
submitted in conformity with the requirements for
the degree of Master of Science by the University
of Alberta.

By
Marian K. Lipkind

Department of Biochemistry
University of Alberta
March, 1940

Acknowledgement

I wish to thank Professor G. Hunter for his interest and advice; and Dr. L. B. Pett for his supervision and assistance in this investigation. The vitamin A tests done by Miss A. Revell, Infirmary Nurse, are greatly appreciated.

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PREFACE

Vitamin A deficiency is associated with changes in epithelial tissues in the body, wherever they occur. Thus it affects the skin, the liver cells, the gastro-intestinal tract and the eye. Night blindness or sub-normal dark adaptation of the eye is an early symptom of a lack of vitamin A. Attempts to measure the visual purple regeneration form the basis for many tests for the diagnosis of vitamin A deficiency.

The above mentioned tests depend upon the bleaching of the visual purple during exposure to a bright light. Since vitamin A enters the composition of visual purple (rhodopsin) in the rods, the rate of resynthesis of rhodopsin depends at least partly upon the available supply of vitamin A. Therefore, if there is sufficient vitamin A present the recovery time or regeneration time will be short. However, if there is a deficiency of vitamin A in the rods, the recovery time will be long.

Many factors may affect the visual test for A deficiency. Instruments purporting to measure this deficiency have not previously been studied from all angles, and thus are not as useful clinically as they might be. It is therefore, the purpose of this investigation to examine the main factors affecting the accuracy of measurements of visual purple regeneration, with especial regard to the assessment of the Pett instrument as a means to this end.

The factors studied fall under the following headings:

I. Conditions of the Test

A study of conditions outside the apparatus and conditions within the apparatus is included here. In studying the factors outside the apparatus, an investigation was made of (1) the conditions of the subject's eyes; (2) the effect of drugs on the eyes; and (3) the effect of light at the headpiece of the instrument. In a study of the conditions within the apparatus the effect of variations in exposure of the eyes to the bright light is investigated. This variation in bright light exposure may be brought about by varying the intensity of the bright light, or the diration of the exposure time to the bright light.

II. Psychological Factors.

These include the study of the learning capacity of the individual with repeated tests. A practice effect--tests gradually getting shorter--has been observed, if a small number of tests was made at very short intervals.

III. Dietary Factors.

Which include the study of

(1) the previous dietary history of the subject,

(a) seasonal variations

(2) the effect of intake of vitamin A or its precursors, and

(3) the diurnal rhythm.

IV. Blood analysis is another method of testing for vitamin A deficiency. However, very little study of this procedure has as yet been made.

INTRODUCTION

The return of vision after blinding by a bright light (and the adaptation of the eyes to darkness) depends upon the ability to regenerate retinal visual purple from vitamin A. Several reviews have built up this hypothesis. Tansley, 1931, reported the effect of vitamin A in the development and regeneration of visual purple in the retina. Hecht, 1937, pointed out the mechanism of the rods and cones in the chemistry of the visual cycle. Jeghers, 1937, correlated vitamin A deficiency and night blindness. Wald, 1937, showed the part played by carotene and vitamin A in the visual cycle, and Booher, 1938, reported the vitamin A requirements for the prevention of night blindness. Various instruments for testing dark adaptation have been used. In the earlier days Augbert's glowing wire, of 1865, and the Birch Hirschfeld Photometer 1916. More recently we have other instruments, Tscherning 1924, Edmunds 1925, Feldman 1934,and 1937, and Jeans and Zentmire 1934 and 1937. Jeans and Zentmire call their instrument the "biophotometer".

The Birch Hirschfeld photometer is rather complicated in construction, and measures the intensity of light required before the subject can distinguish 3 points of light in a

quincunx of unequally illuminated perforations in a black screen. The subject is exposed to a very bright indirect light for 5 minutes, and then left in total darkness for 10 minutes while photometric readings are made at constant time intervals. The complete test takes only slightly less than 20 minutes. This procedure has been rather severely criticized by Snelling 1936 on the basis that it does not estimate small variations in dark adaptation such as might be caused by avitaminosis A.

The Edmund, 1925, procedure is a method of examination for distinction powers. This test requires the subject to differentiate large letters, printed in nine shades of greyness, from a white background--(illumination being controlled by a series of Tscherning photometric glasses). The subject must first be completely adapted in the dark. The test takes a long time, and the illumination of the letters may vary. Although under standard conditions this test may be accurate for experimental studies, it would therefore seem to be unsatisfactory for routine use, owing to the time taken.

The biophotometer, being available commercially, is now one of the chief instruments used. The procedure is very like that of the Birch Hirschfeld photometer, with only slight variations.

The 'visual adaptometer' of Hecht, 1938, is used as follows: the subject is exposed to light adaption for 3 minutes and then darkness. The time elapse before a light of a certain intensity can be seen as well as both rod and cone threshold are measured in this procedure. Each eye is tested separately, which makes the test very long, about one hour being required. Therefore, although this test is experimentally accurate and satisfactory it is not so useful clinically.

Feldman, 1938, devised two 'adaptometers'. The first, a qualitative device, is very complicated in construction. It measures threshold of light intensity over a period of time, and takes at least one hour for a complete test. The other instrument, a quantitative apparatus, measures the time for a given threshold of light at fixed intensity after a 3 minute light-adaptation period. Both the above procedures test each eye separately.

Severe criticisms of the Biophotometer, especially regarding its ability to detect vitamin A deficiency have been advanced; (Gridgeman and Wilkinson, 1938; and Isaacs, Jung and Ivy, 1938.) They find:

1. A considerable learning factor - improvement in photometric readings with experience.
2. The illumination control is inaccurate-- An insufficiently sensitive device for cont-

rolling test illumination. Only crude approximations can be made at the bright end, and this is where most hemeralopic readings occur.

3. Some uncertainty and difficulty in establishing the normal.
4. Errors are introduced by changing photometric readings into millifoot candles.

The latest review of the dark adaptation test,(Harris and Abbasy, 1939), supports the principles of the Biophotometer. However, they have gone back to the procedure for the Birch-Hirschfeld Photometer, and report it satisfactory for routine use.

On the whole the instruments mentioned above appear to be very complicated in construction, seem to require practiced subjects, and all take considerable time for a test. The Feldman and Hecht Adaptometers, although possibly the most accurate experimentally of all the procedures, are not so useful clinically because of the length of time taken for each test.

The apparatus used in this investigation has been described by Pett (1939) as follows:

> "The instrument here outlined consists of a black wooden box, about 1 cubic foot in volume. Protruding from the front is a headpiece, and on the back are various instruments as seen in Figure I.

PICTURE OF MODEL VII

Inside the box are: (1) a source of bright white light for bleaching visual purple in the eyes, coming from either one or several bulbs; (2) a surface for reflecting this bright light into the eyes while looking into the headpiece, such that 50 foot-candles is the intensity at the eyes; the bright light is connected to a voltmeter and rheostat, and each instrument is calibrated by a photometer to operate at a certain voltage, the reflecting surface is paper (English Cartridge Paper, 100 pounds), is 12 inches square, and covers a metal shield from which two rectangles 3/4 inch by 3/16 inch are cut out; (3) behind the metal shield is a dim light, so placed as to shine evenly through the two rectangles. In passing through the paper this light loses ninety percent of its intensity and at the eye has an intensity of about 4 milli-foot candles, and is slightly reddish. A mechanical device controlled from the back of the box is used to cover one of the rectangles of dim light, while the other remains visible, the subject then having to distinguish between the vertical and horizontal directions."

In the procedure for the test "the subject adjusts his head comfortably against the rubber forehead rest, wearing glasses if they are usually worn, and is shown the rectangles of dim light, one 'Up', one 'Across'. The bright light is put on, and he is asked to look at a black spot marking the position where the bar of dim light will later appear when the light goes

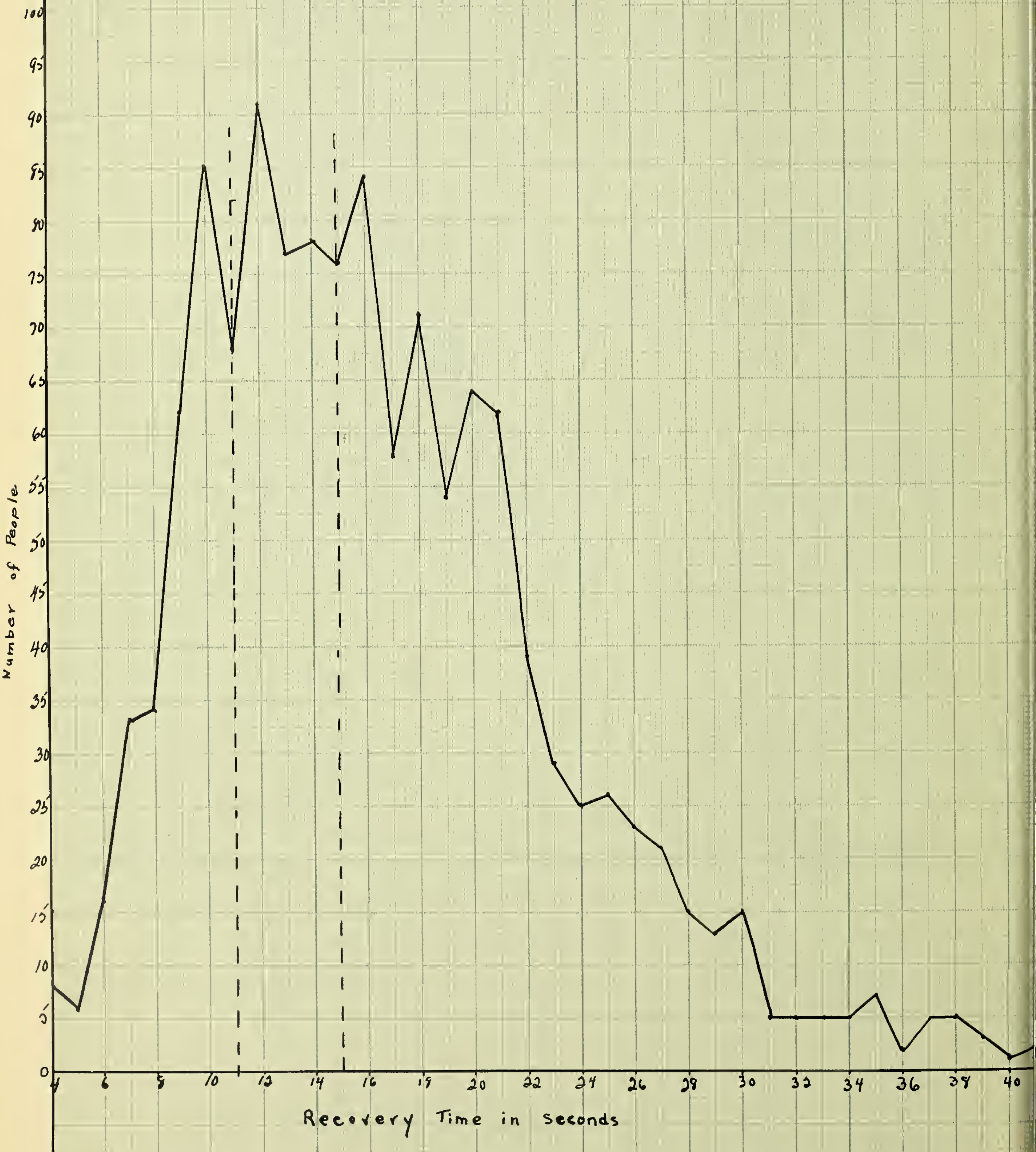
> out. During this period of thirty seconds, the name, date, etc., can be recorded on a card and the voltage checked. As the watch hand crosses thirty seconds the bright light is turned off, and the subject is reminded to say "UP" or "ACROSS", indicating the direction of the rectangle seen, as soon as the direction can be made out. The time is noted when this is said, and represents the recovery time from thirty seconds of standard bright light".
>
> "Tests are repeated at minute intervals, three usually sufficing. In some cases the three values may be rather divergent, especially when the first value is long. It is then considered more accurate to use the median or mid-value, rather than the mean; e.g. of three values 23, 15, 16, use the value 16 rather than the mean 18. The range of recovery times is from three to sixty seconds, with a mode of eight".

The first machine (Model I) was built by Pett in 1937 and since then seven other models have been made. The fundamental principles of construction were settled by experimentation prior to making Model IV in the fall of 1938. Subsequent Models have differences only in the housing of the apparatus.

In working in this field several standards are necessarily arbitrarily chosen. One of these was the standard set for the dividing line between "normal" and "deficient". In the beginning of this work, it was decided that any person with a "recovery time" of over 11 seconds was "deficient" in vitamin A, and the longer the "recovery time", the greater the deficiency. Although this division is not correct in all cases, it was substantiated by Pett (1939) as follows: (1) In plotting the frequency distribution of 1,519 people, a pronounced dip at 11 seconds suggested a bimodal curve, (a combination of two curves each with its own mode). The two curves

Figure II

Frequency Distribution Curve



would be: (a) distribution of people normal in vitamin A; (b) people deficient in vitamin A. Frequency distributions of 1600 cases have been plotted during this investigation. Figure II shows these frequency distributions plotted, and the characteristic dip at 11 seconds. 200 persons called "deficient", (recovery times longer than 11 seconds) were treated with vitamin A. As a result, recovery times were shortened to 11 seconds or less. Thus these people were shifted from the "deficient" group to the "normal" group.

The work just reviewed, and the present thesis, show that the Pett apparatus has the following advantages over the previously mentioned instruments for measuring vitamin A deficiency.

1. It is the least complicated in construction of any of the instruments.
2. If the test is done as prescribed, there is no "learning" or practice effect.
3. Subjects need not be educated previous to the test.
4. The complete test is very rapid--between thirty and forty persons an hour have been tested by one operator with only one machine.
5. This procedure is subjected to fewer errors of all kinds.
6. This is the only test for vitamin A deficiency which has been correlated with blood analysis for vitamin A, (Pett and LePage, 1940).

7. Although many of the other procedures for measuring vitamin A deficiency may be as accurate as that of Pett, the Pett procedure is the only one which has been analysed and proved accurate from a statistical point of view.

EXPERIMENTAL

I. Conditions of the Test

A. Factors Outside the Apparatus

1. Condition of the subject's eyes.

In studying conditions of the subject's eyes, it was found that no effect is produced by removing glasses if they are usually worn. Glasses if worn by the subject are usually retained in the test for the sake of speed. A study was made on 12 persons by testing with glasses on, and retesting with glasses off after one half hour interval in which the eyes adapt themselves to the change. The results are shown in Table I.

Table I

Recovery Times of 12 Persons Tested With Glasses On, and Retested After One Half Hour With Glasses Off.

Subject	Test With Glasses On	Test With Glasses Off
(1)	15	16
(2)	15	16
(3)	12	10
(4)	9	8
(5)	8	7
(6)	10	10
(7)	5	6

Table I (cont'd)

Subject	Test With Glasses On	Test With Glasses Off
(8)	11	10
(9)	10	9
(10)	11	10
(11)	11	12
(12)	10	11

In the 12 cases studied no significant differences were found between the two tests.

Ten other cases were studied in which there was a 5 minute interval between tests with glasses on, and glasses off. The results are summarized in Table II

Table II

Subject	Test With Glasses On	Test With Glasses Off
(1)	14	14
(2)	13	11
(3)	27	27
(4)	21	18
(5)	21	23
(6)	12	13
(7)	12	13
(8)	11	13
(9)	17	19
(10)	10	14

The differences between tests with glasses on and off are in one or two cases greater than tests with one half hour interval. Subject (10) shows an appreciable and even significant difference between the two tests, but it is the only case in ten. This may be explained by the fact that the eye cannot completely adjust itself to the change in five minutes. The eye is adjusted after one half hour, and therefore the tests after this interval are practically the same.

2. The effect of drugs on the eye.

The effect of drugs was studied on six persons who received 2 drops of 2% Pilocarpine Nitrate in each eye. Each person was tested previous to the administration of the drug, and again one hour and two hours after. The test one hour after the pilocarpine, (which causes the pupil to contract) was given, was greatly prolonged, and even after two hours, the test did not resemble normal, although it was much shorter than the first. Results are shown in Table III.

Table III

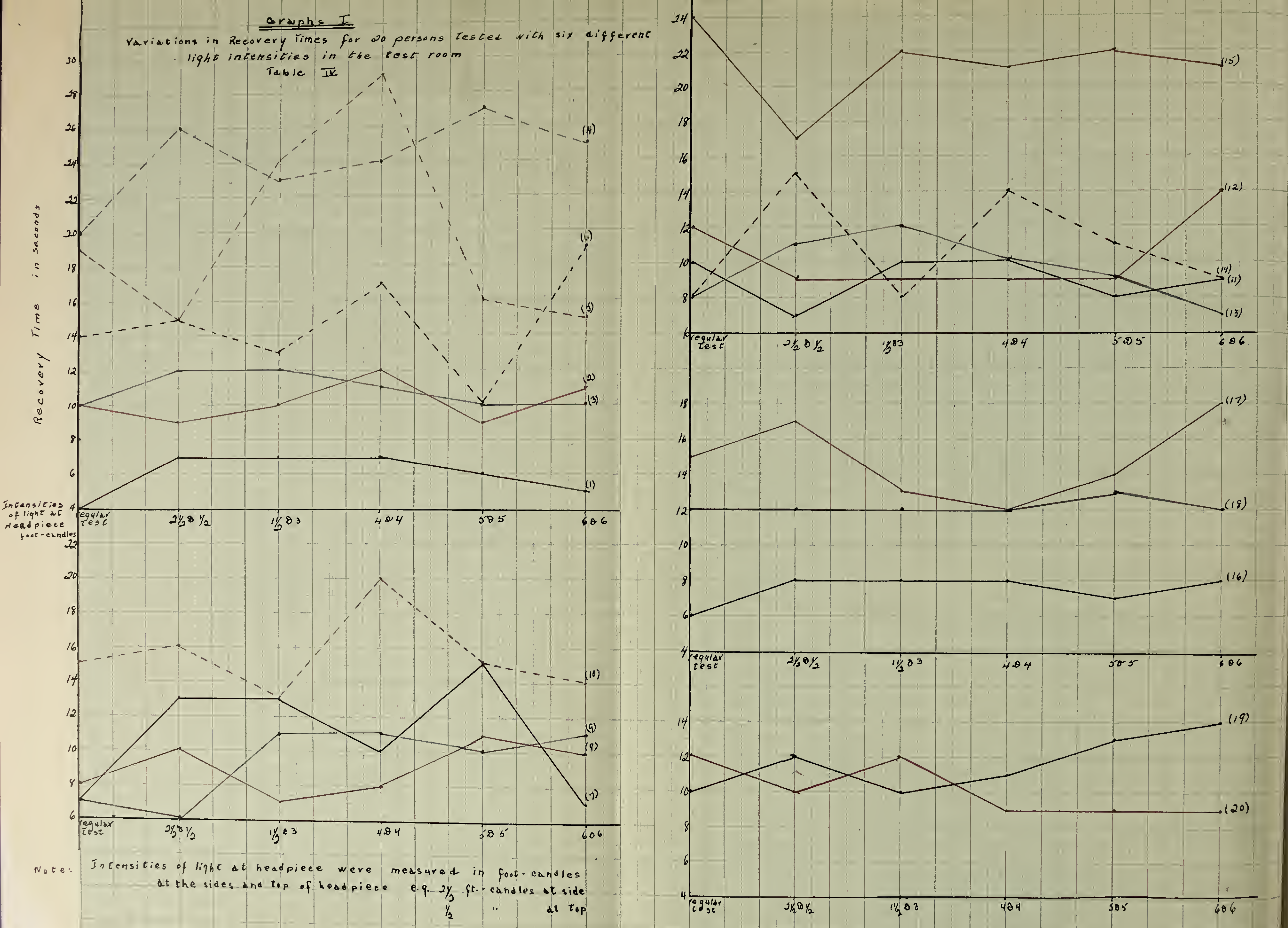
Recovery Times of 6 Persons Before and 1 and 2 hours After the Administration of 2 Drops of 2% Pilocarpine Nitrate

Subject	Normal Test	Test 1 Hr. After Pilocarpine	Test 2 Hr. After Pilocarpine
(1)	12	45	16
(2)	12	57	24
(3)	6	18	11
(4)	9	77	32
(5)	16	80	33
(6)	9	24	18

Feldman, 1939, has advocated instilling pilocarpine nitrate into the eyes before a test in order to assure full contraction of the pupils. We find this procedure unsatisfactory for three reasons: (1) it prolongs the recovery time; (2) it distresses the subject; (3) the effect of the drug begins to wear off as the eyes finally adapt themselves.

(3) The effect of Variations in Intensity of Light at the Headpiece.

The effect of variations in intensity of light at the headpiece of the Pett machine for testing vitamin A deficiency has been studied on 20 persons. The light variations



Graphs I

Variations in Recovery Times for 20 persons tested with six different light intensities in the test room

Table IV

Note: Intensities of light at headpiece were measured in foot-candles at the sides and top of headpiece e.g. 2½ ft.-candles at side
½ .. at top

were obtained by allowing varying amounts of light into the test room. The intensities of light vary from one and one-half to six foot-candles, (at the headpiece), and tests were made on each person at each of six variations. The results are tabulated in the Appendix, Table IV.

By plotting recovery times against headpiece intensities, Graph I was obtained, the study of which led to the following conclusions. The best light range from one and one-half to six foot-candles differs somewhat with the individual. Therefore, a small amount of light may be let into the test room providing it be kept constant for all tests on the same person. Also the test room usually dark, but not completely dark, is best kept that way for routine testing.

B. Factors Inside The Apparatus.

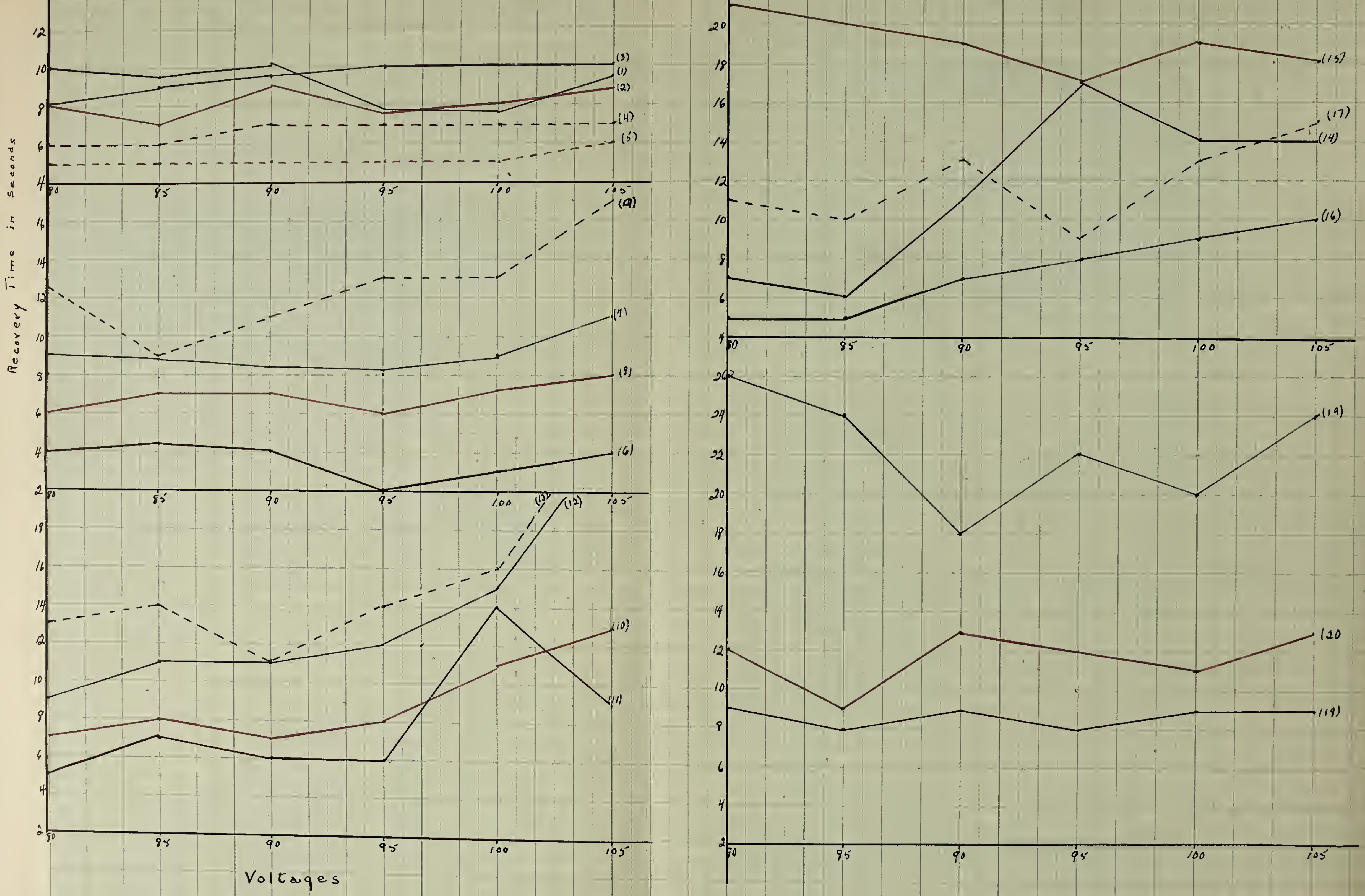
1. The effect of variations in exposure of the eyes to the bright light exposure may be brought about by varying (a) the intensity of the bright light; (b) the duration of the exposure time to the bright light.

(a) The effect of the variation in intensity of the bright light was studied on 20 persons. The bright light was set at six different intensities by changing the voltage. Tests were taken on each person at each voltage--80 volts, 85

Graphs II

Variations in Recovery Times for 20 Persons with different bright light Intensities – brought about by changing Voltages

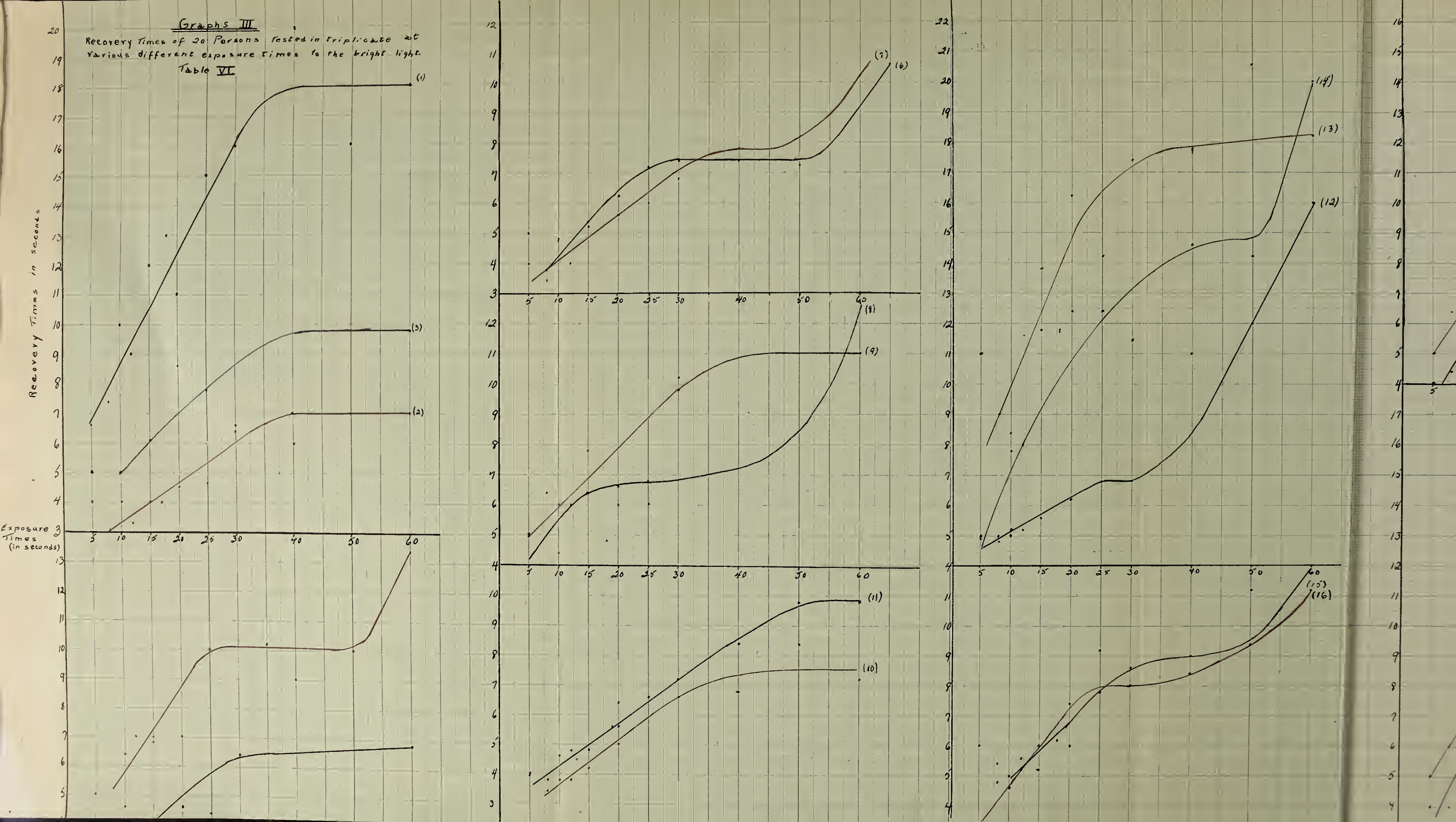
Table V



volts, 90 volts, 95 volts, 100 volts and 105 volts. The results are tabulated in the Appendix--Table V.

Graph II was drawn, in which the recovery times were plotted against voltage. From examination of these curves, we may conclude that there is a voltage range where most consistent results are obtained. This optimum range in voltage is from 85 to 100 volts. Recovery times at voltages below 85 volts seem to be extremely variable, and increase with voltage increase. That is presumably because bleaching by a light of low intensity does not bleach the visual purple to a constant value, and therefore recovery time is not constant. If the light is brighter the visual purple is bleached to a constant value, and the recovery time is constant. Also, at voltages above 100 volts the recovery time is sharply prolonged, probably due to the appearance here of a fatigue factor.

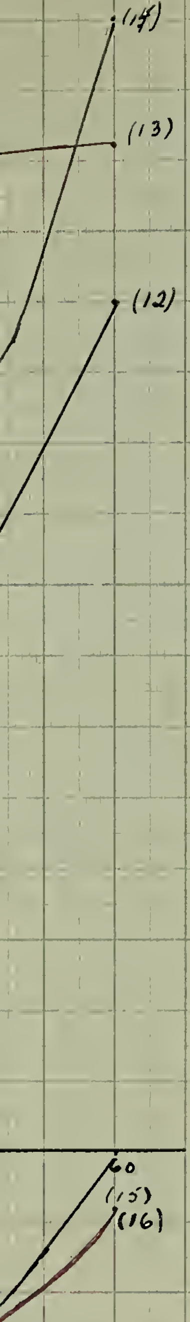
2. The effect of changing the duration of exposure time to the bright light gives results somewhat similar to the above. Twenty cases were studied, and exposure times varied from 5 to 60 seconds. Tests were taken on each person both in triplicate and single tests at each exposure time. The results are summarized in the Appendix--Tables VI and VII.



Graphs III

Recovery Times of 20 Persons tested in triplicate at various different exposure times to the bright light.

Table VI.


(14)
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(15)
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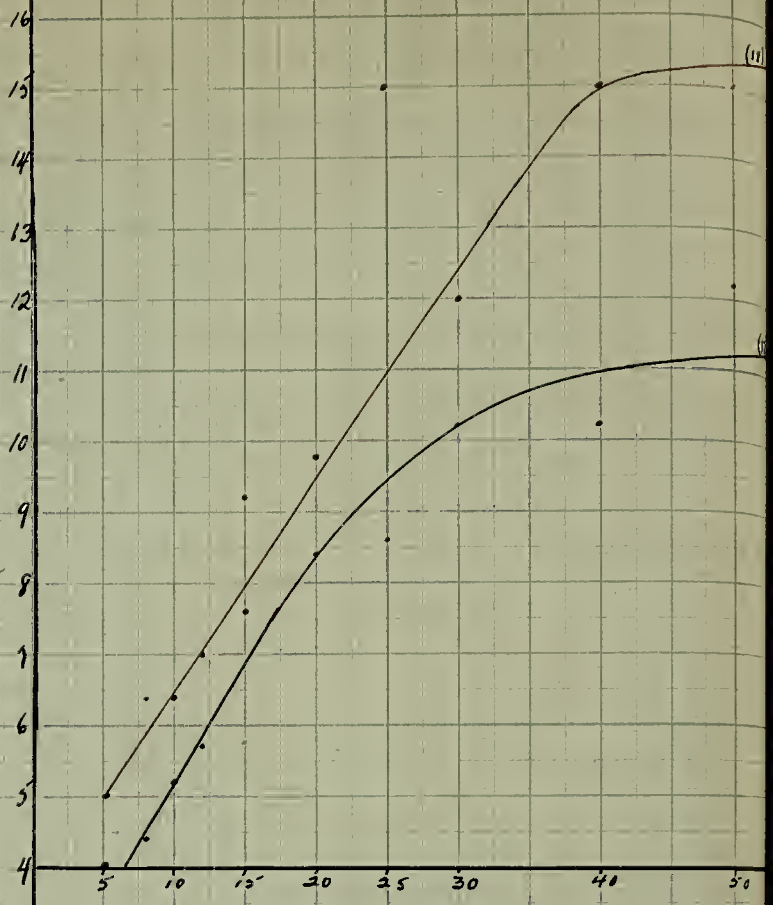
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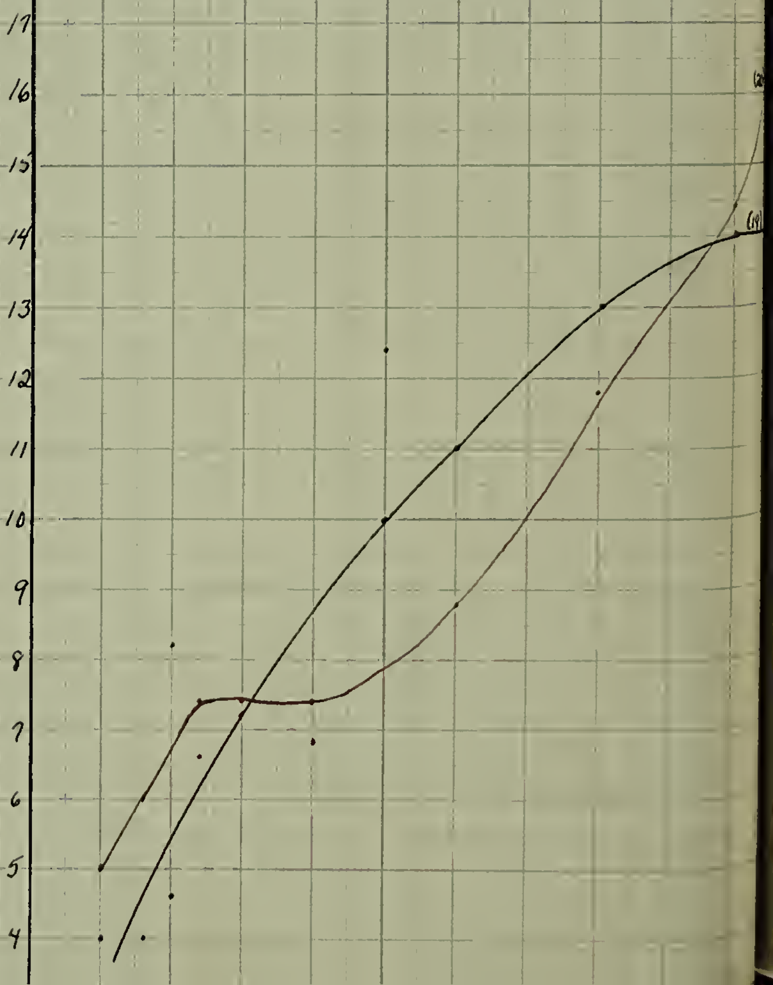
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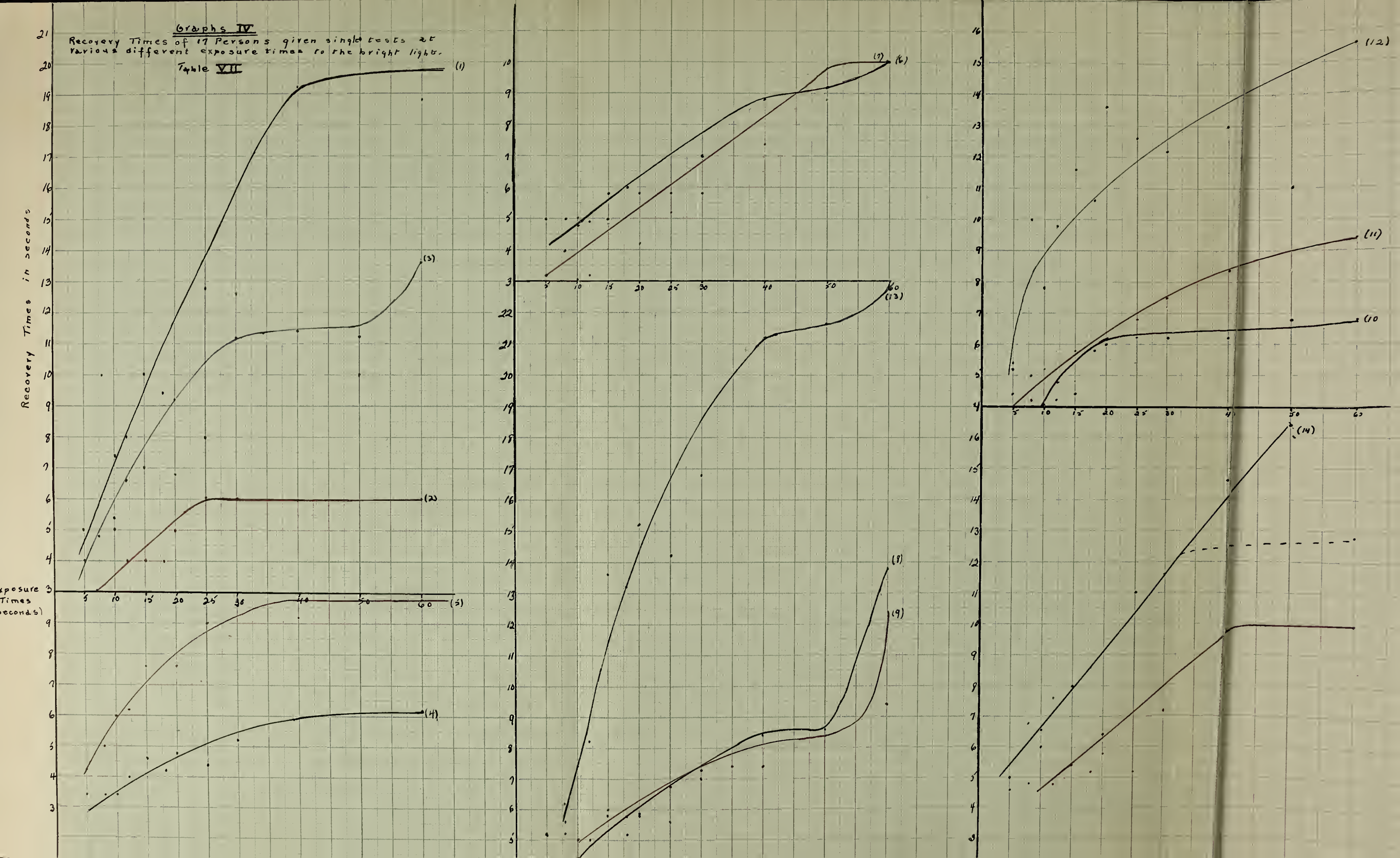
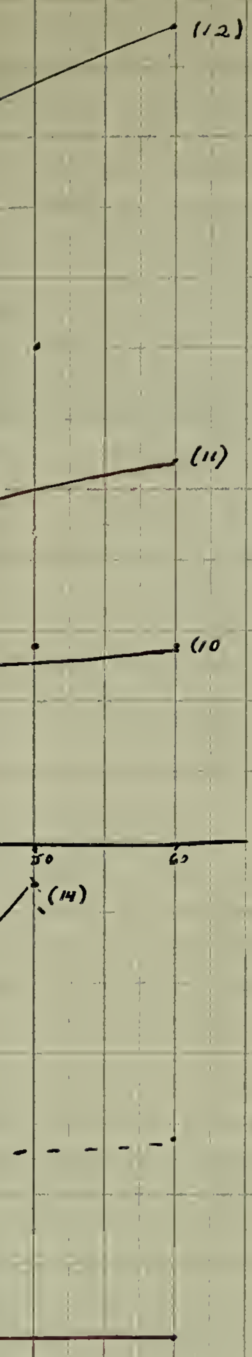
Graphs IV

Recovery Times of 17 Persons given single tests at various different exposure times to the bright light.

Table VII

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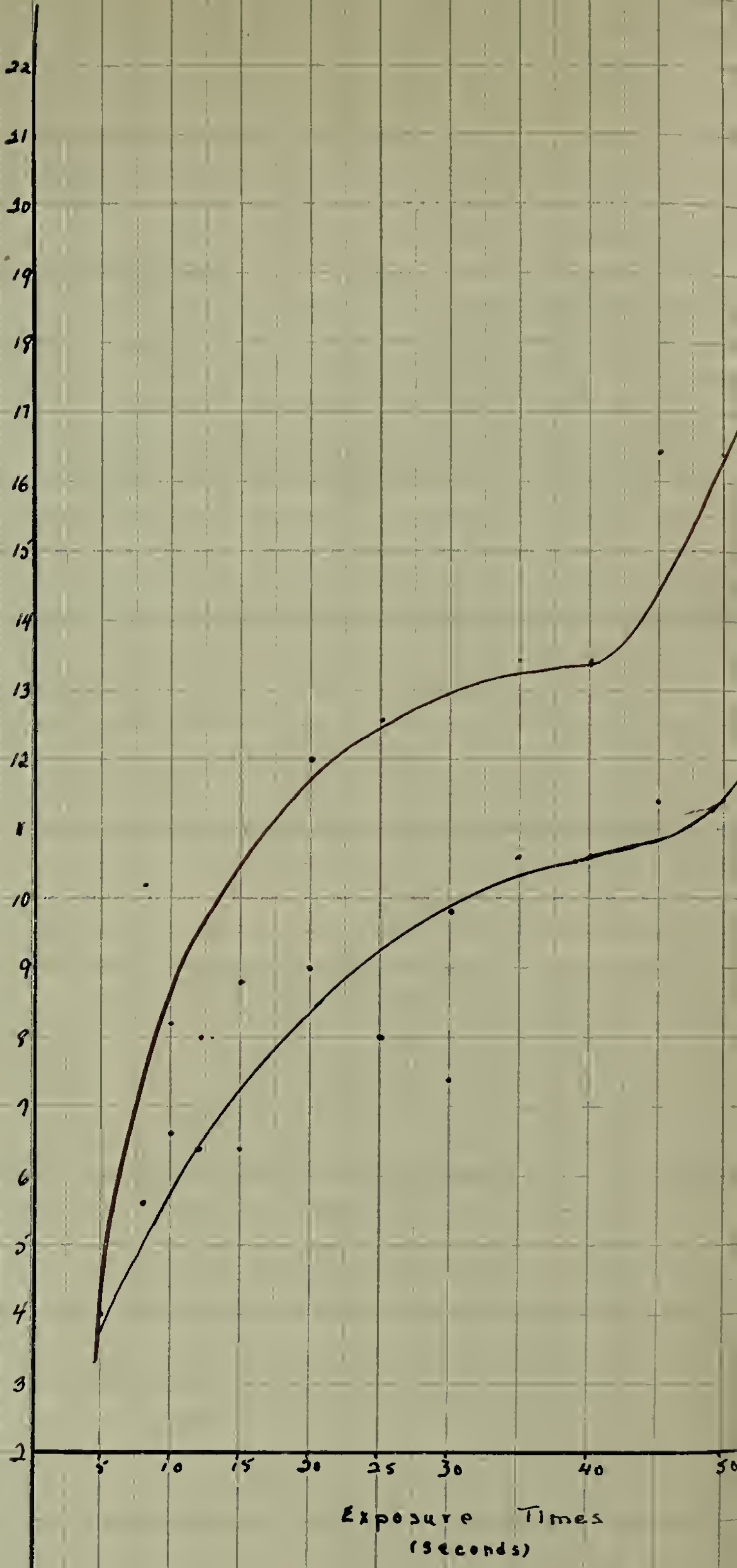
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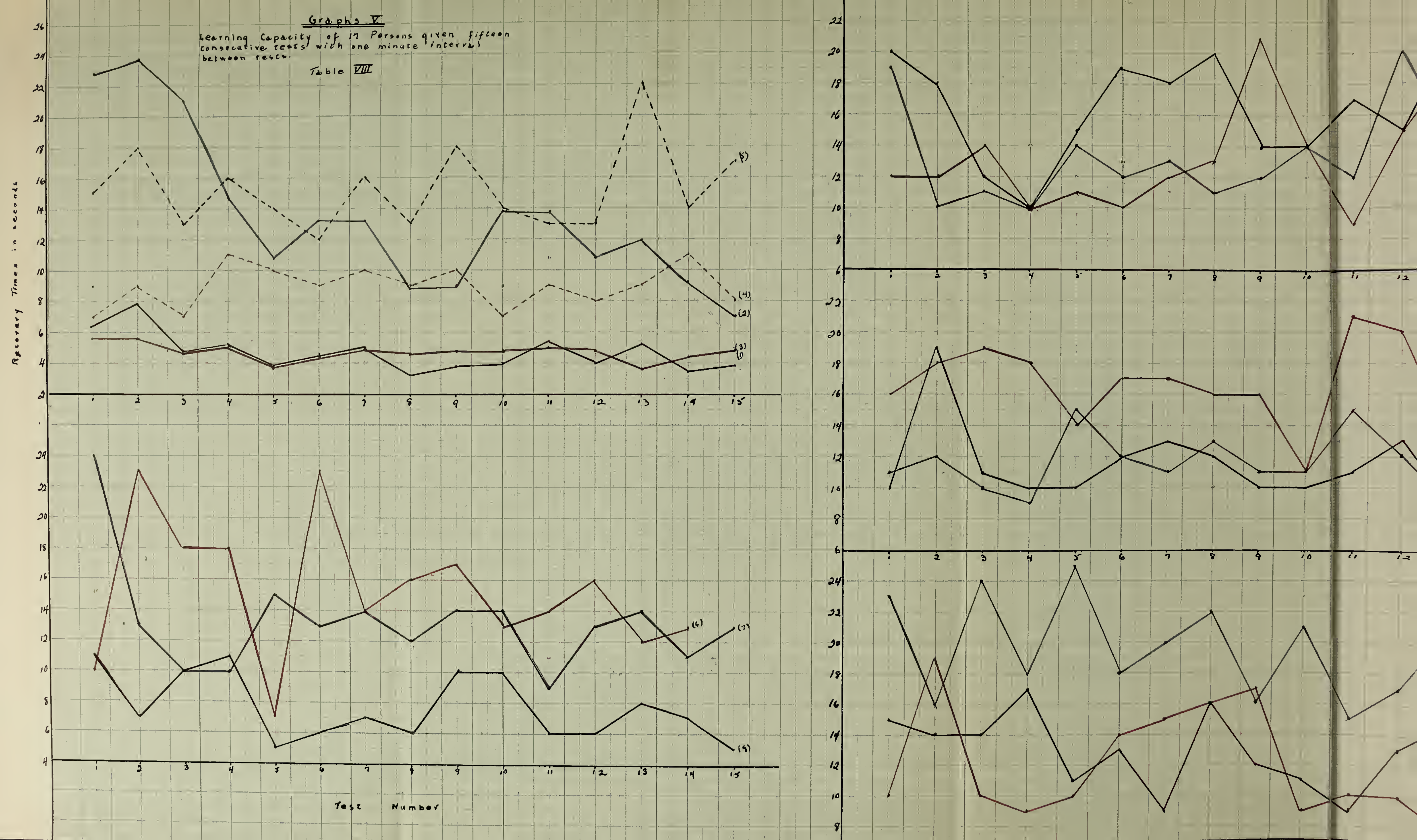
50
Exposure Times
(seconds)

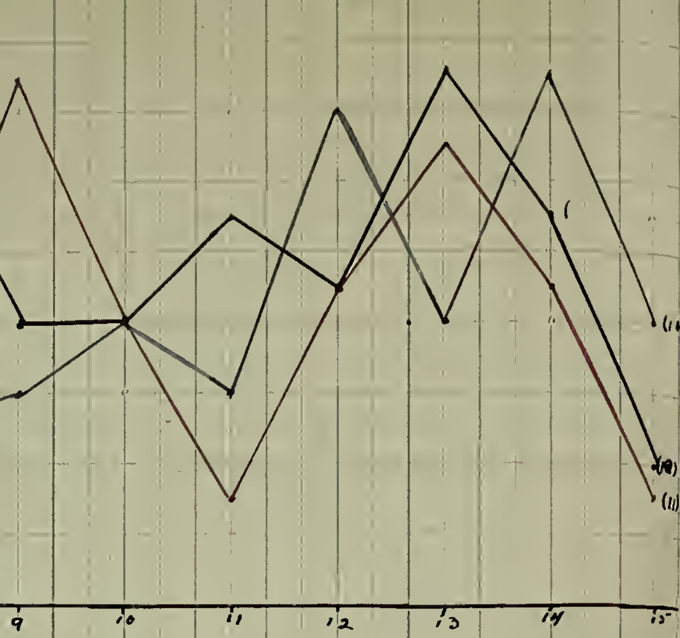
Graph III was obtained by plotting recovery times against exposure times. The two resulting curves corresponding to the triplicate and single test procedures for each person (Graph IV) were similar in almost every case. By studying these curves we found that the range in exposure times from 25 to 40 seconds, gave the most consistent results. Moreover, recovery times taken at exposure times below 20 seconds varied a great deal with the individual, and increased with the exposure time increase. This, presumably, depends upon the fact that when the exposure time to the bright light is short, the visual purple is not bleached to a constant value, and therefore the recovery time is not constant. However, when the exposure time to the bright light is increased, the visual purple is bleached to a constant value, and the rate of recovery and hence recovery time becomes constant. It was also found that when the exposure times were above 40 seconds, we had the important fatigue factor to contend with, and recovery times were very irregular and prolonged.

Graphs V

Learning Capacity of 17 Persons given fifteen consecutive tests with one minute interval between tests.

Table VIII





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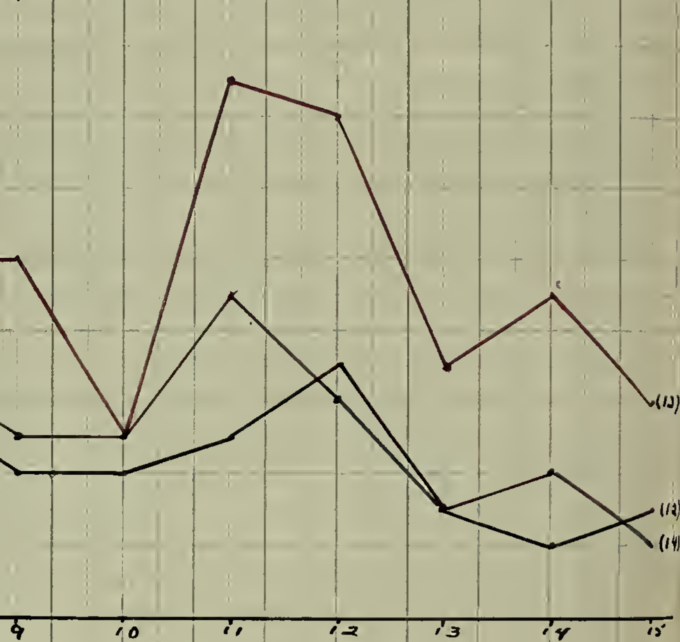

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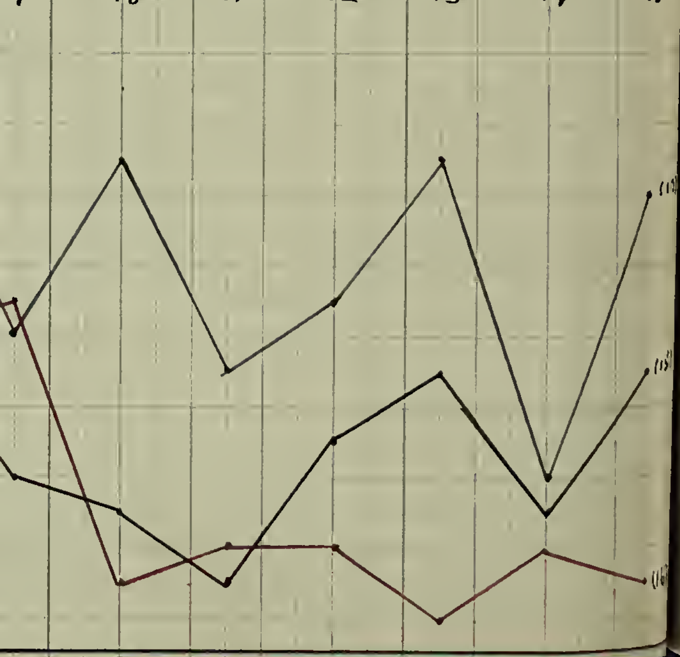


(17
(15
(16

II. Psychological Factors.

This section includes a study of the learning capacity of the individual with repeated tests. A practice effect (tests gradually getting shorter) has been observed if a small number of tests were made at very short intervals. However, if a large number of tests were done, or the interval between tests lengthened, this effect disappeared.

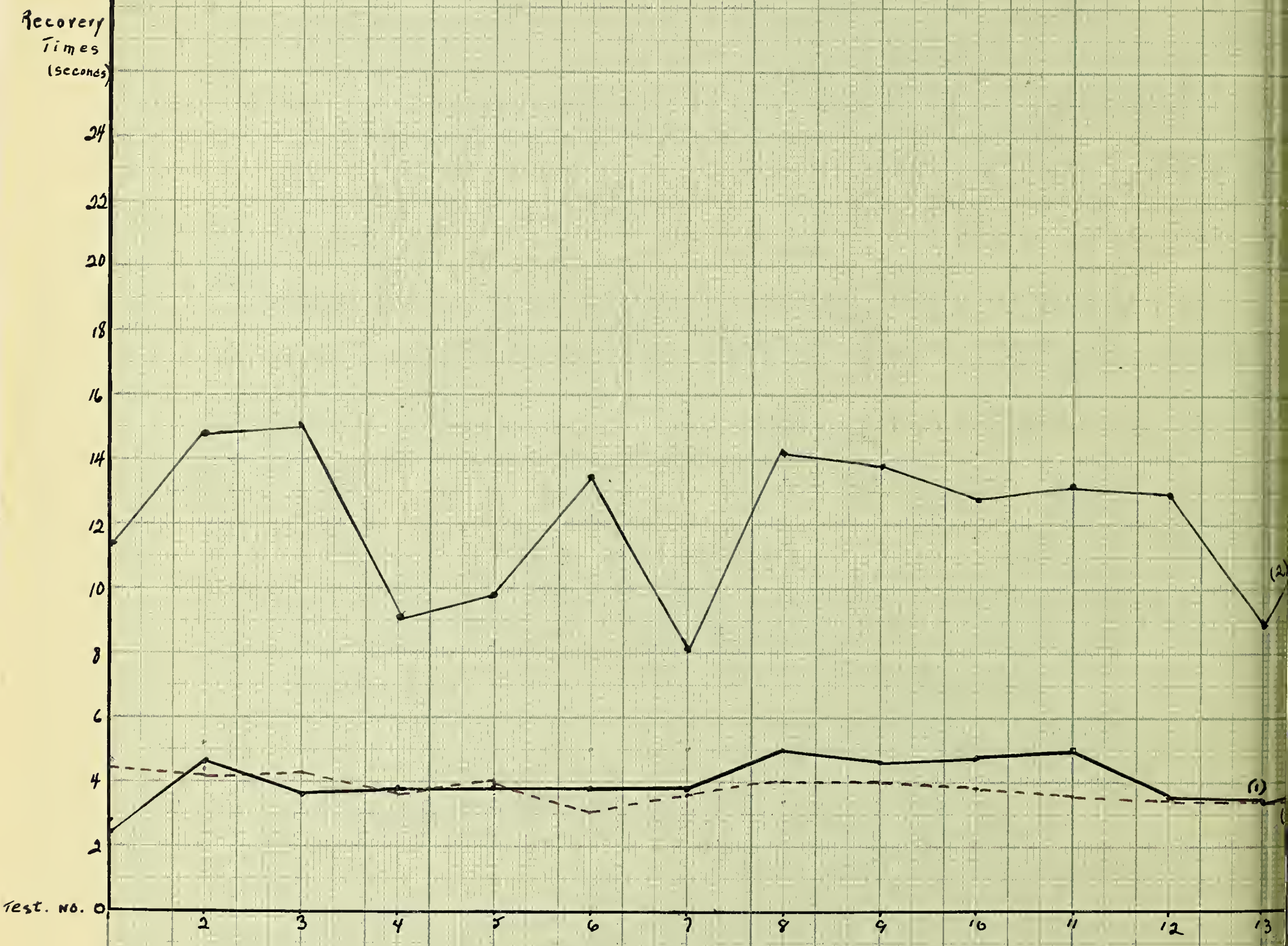
A study was made on 17 people, giving each person 15 tests with only one minute interval between tests. The results of this investigation are summarized in the Appendix, Table VIII. Examination of the results, show that only one subject (2) exhibits any tendency towards learning with repeated tests. Graphs were made by plotting recovery times for each test. Graph V, plotted for subject (2) showed the only learning curve in the 17 cases. Also, it was found that by lengthening the interval between tests--say from one minute to five minutes, no learning tendency is shown. Table IX in the Appendix gives the results of this procedure, and Graph VI, plotted as above, shows no learning curves. Even subject (2) does not show a learning curve ~~with~~ when the interval between tests is lengthened from one to five minutes.

A study was also made on 25 persons receiving a single test a day (not the prescribed 3 tests averaged) for 21 days. The results are shown in

Graphs VI

Recovery Times of 3 Persons given fourteen consecutive tests with a five minute interval between tests.

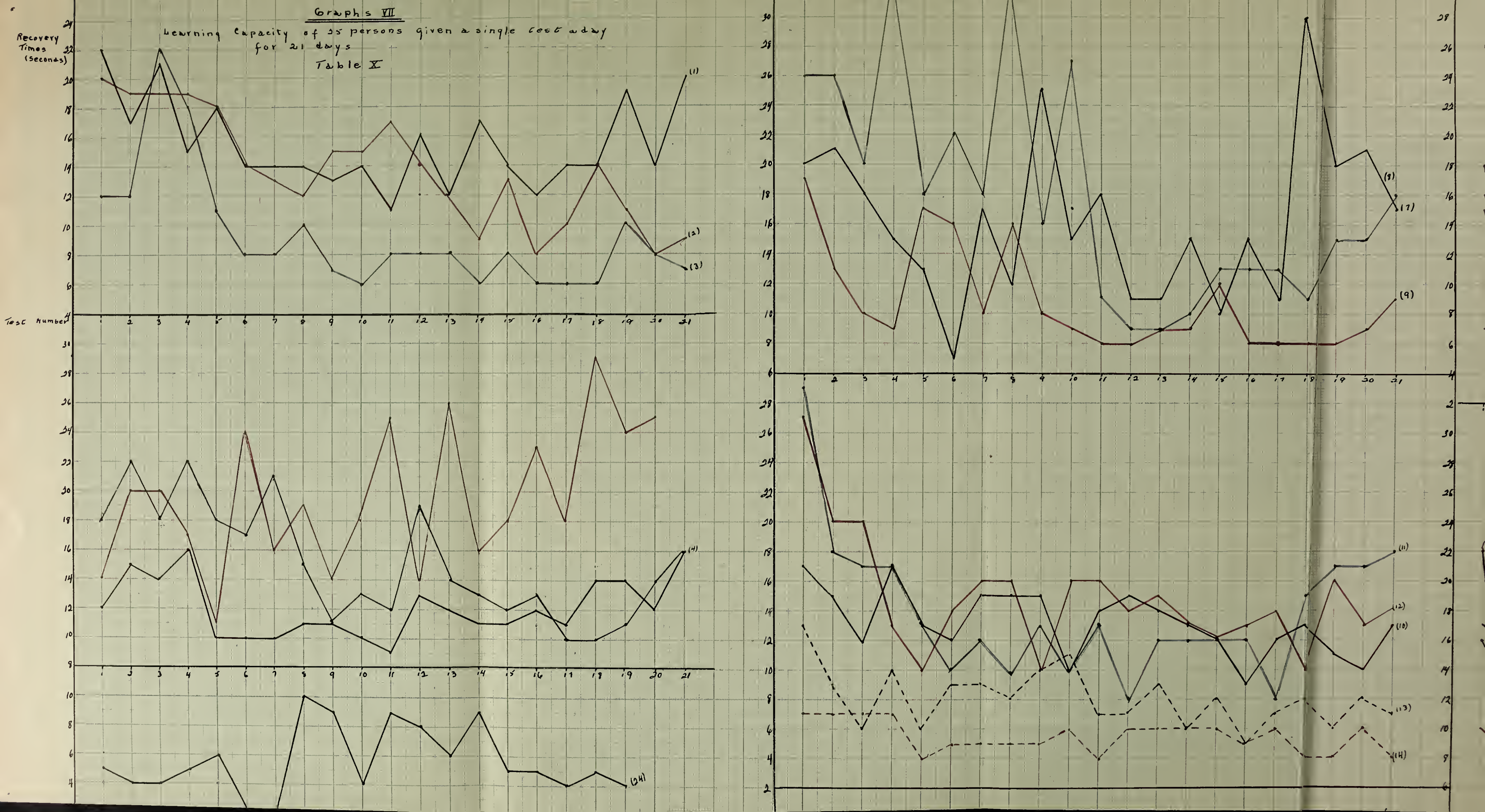
Table IX



Graphs VII

Learning Capacity of 35 persons given a single test a day for 21 days

Table X



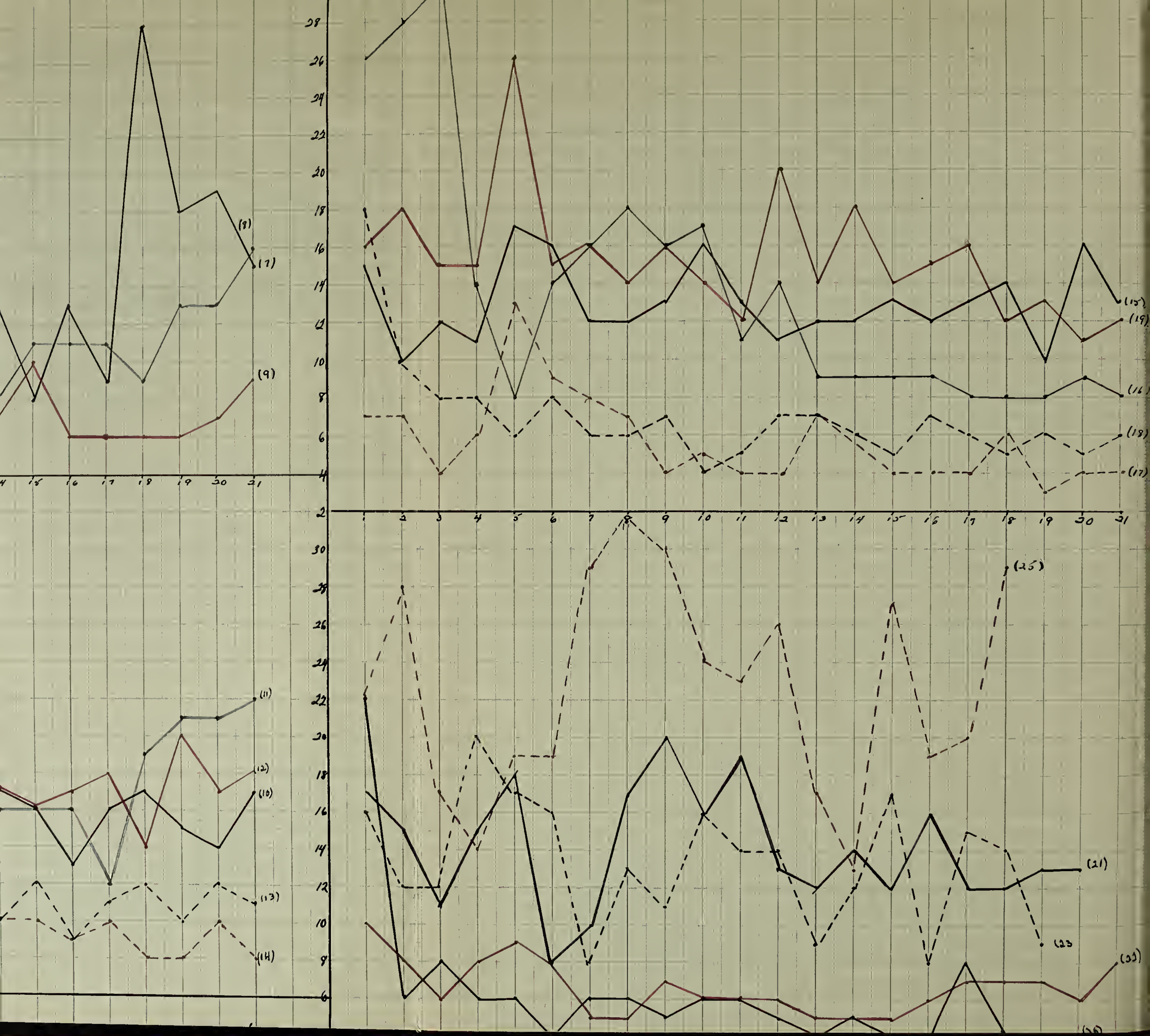

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Table X in the Appendix. Graph VII, obtained ~~in a similar manner~~ as above, showed learning curves in 11 cases or 44 percent of the cases, (Subjects (2), (3), (6), (8), (9), (11), (12), (13), (16), (18), (20)). However, in a similar study of 10 cases, but doing the prescribed three tests at a time every day, no learning curves were obtained,--Graph VIII. The results are shown in Table XI in the Appendix.

Although repeated tests, e.g. in following a course of treatment or etc., may sometimes show a practice effect, this is too rare to be of serious consequence.

The above conclusions may be verified from mathematical treatment of the data obtained. The Standard Deviation with its plus-and-minus sign marks off a small distance above and below either the mean or the median. The larger the distance, the less accurate is the mean or median. The deviations from the average (mean), σM, and from the Median, σMe, are given for:

1. The experiment in which 17 persons were tested 15 times at one minute interval.
2. The experiment in which 25 persons were tested once daily.
3. For every twentieth person in 820 persons given the prescribed test.

In the Appendix, Table XIII (at end of Table XI) shows the detailed comparisons of the Mean Deviations for these three experiments.

Graphs VIII

Recovery Times of 10 Persons given the prescribed test every day for several days.

Table XI

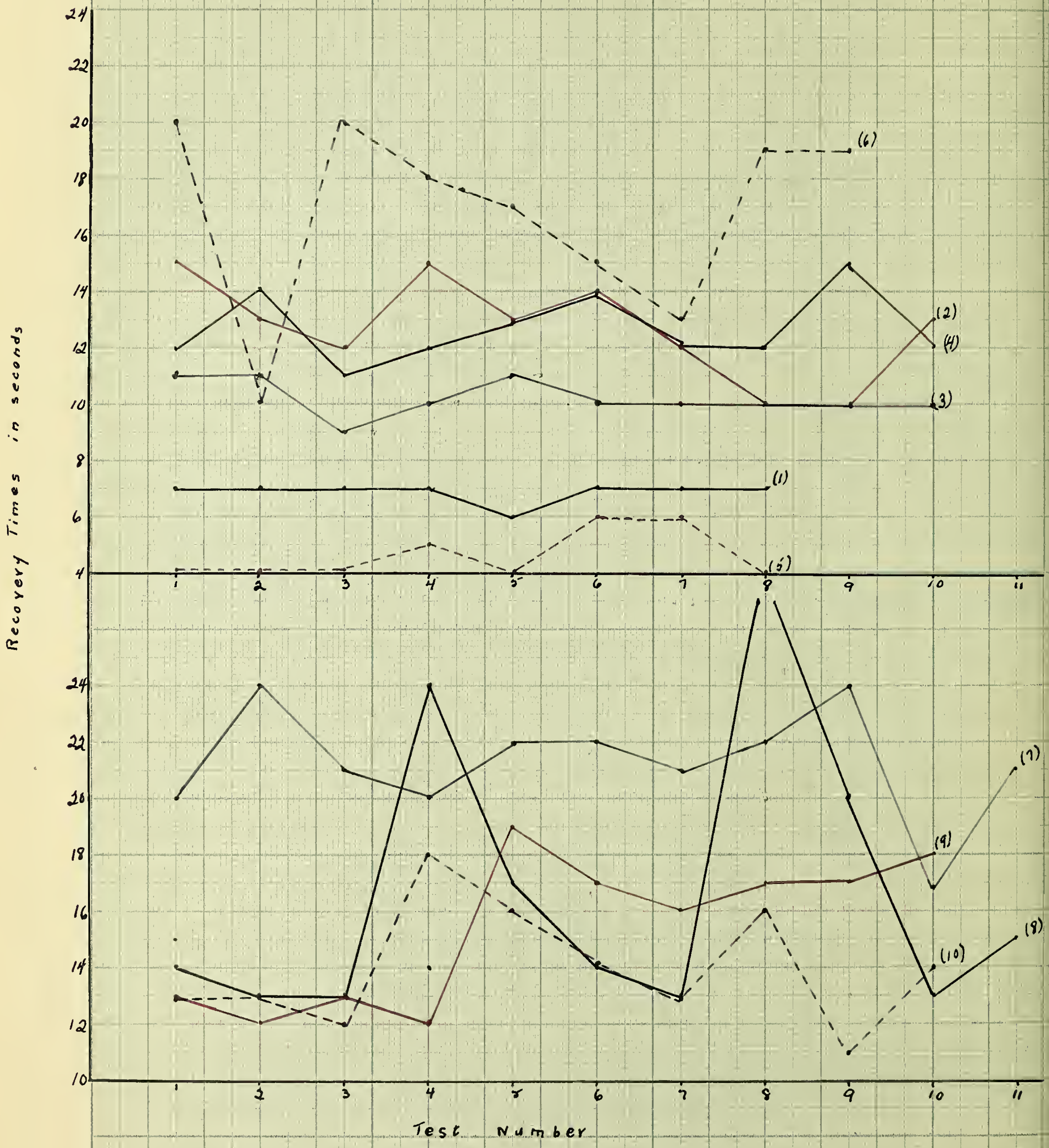

Table XII gives the comparison of the σ_{Me} and σ_M deviations alone for the three experiments.

Table XII

Comparison of Mean Deviations For
Experiment (1), (2), and (3)

σ_M sec.	σ_{Me} sec.	Experiment
±2.5	±4.5	(1) 15 consecutive tests on 17 persons-"learning" shown in 1 out of 17 cases.
±3.5	±5.6	(2) One test daily on 25 persons--"learning"shown in 11 out 25 cases.
±2.5	±4.7	(3) Survey of tests done in recommended manner--"learning" negligible.

Experiment (2) shows more dispersion about the mean, and so is less accurate than experiment (1). Experiments (1) and (3) show the same dispersion about the mean, and hence the same degree of accuracy. This close agreement between the mean deviations for these two experiments, substantiates the conclusion that "learning" is not shown if several tests are done, or if the prescribed three tests are given.

The values obtained here for σ_{Me} are very little higher than those theoretically accurate for the corresponding values for σ_M given in Table XII.

III. Dietary Factors

1. Previous Dietary History.

Tests were done on 1600 cases composed of summer school students and freshmen at the University of Alberta. The tests were done shortly after the arrival of the above students, so that the effect of their previous diet had not been replaced by that of their new status. The summer school students, mainly teachers, were from all parts of the province of Alberta, with a large percentage from Northern Alberta. Table XIV presents the results of the 1600 cases studied.

Table XIV

Percentage of Persons in Both Groups Found To Be Normal (not Deficient in Vitamin A), Mildly Deficient, Very Deficient and Extreme Cases.

Group	Total	% Normal Times (3-11 inc.)	% Mildly Deficient (11-15inc.)	% Very Deficient (16 -)	Extreme Cases
All Cases Studied	1600				
Summer School Students	750	19	25	50	6
Freshmen	850	34	26	38	4

On examination of Table XIV, we find that a much higher percentage of the regular students are either normal or borderline deficients than the Summer School Students. It is suggested that the difference

lies in the previous dietary conditions. The regular student comes from a home of better economic standing, while the majority of Summer School students had had very poor living conditions for months previous to their arrival here.

Each of the above cases was plotted according to the district from which they came, and as a "normal" or a "deficient", on a map of the Province of Alberta.

Deficiencies were found to be more prevalent in certain sections of the province than in others. Examination of the map showed that some relation exists between soil conditions and avitaminosis A. The province is naturally divided into soil belts, Dark Brown, Light Brown, Black, Grey Transitional and Grey. Table XV shows the relationship of vitamin A to the soil belts.

Table XV

Percentage of Persons From Different Soil Belts Found to Be "Normal" in Vitamin A.

Soil Belt	Total No. of Persons	Percentage "Normal"
Dark Brown	233	32
Brown	85	28
Black + Grey Transitional	495	26
Grey	104	23
Peace River	60	32

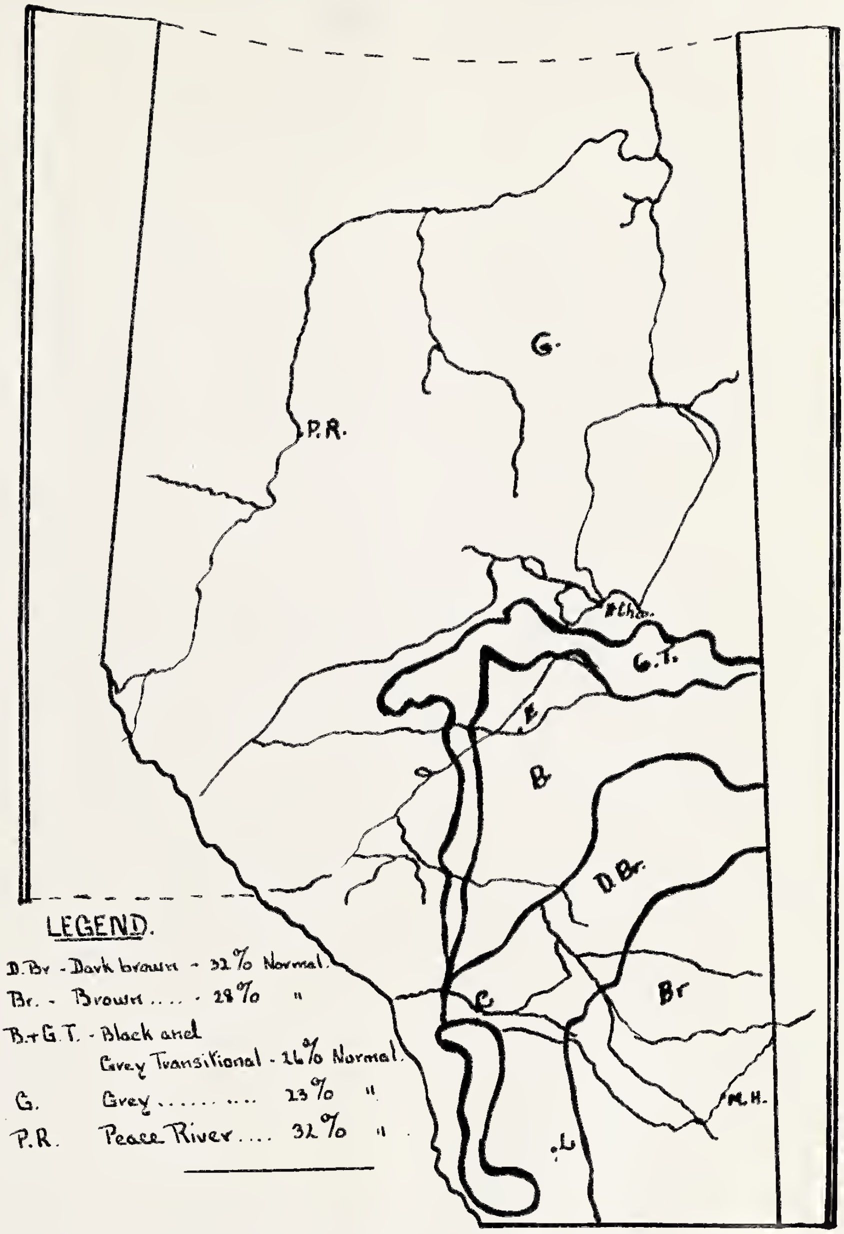
G.
P.R.
Atha.
G.T.
E
B
D.Br.
C
Br
M.H.
L
LEGEND.
D.Br - Dark brown - 32% Normal.
Br. - Brown . . . . - 28% "
B.+G.T. - Black and
Grey Transitional - 26% Normal.
G. Grey . . . . 23% "
P.R. Peace River . . . . 32% "

Figure III - The relationship between soil belts and Vitamin A Deficiency on a map of Alberta.

Figure III shows the above soil and vitamin A relationship on a map of Alberta.

Neither of Alberta's two largest cities, Calgary, 42% "normal", or Edmonton, 37% "normal" have been included in the above Table or Figure.

The Peace River strip of soil is relatively rich and may be compared to that of the Dark Brown belt in fertility. The soil of the Brown belt is poor, and there is probably an error in representation of this area as almost one third of the subjects came from the city of Medicine Hat, and these supplied one half of the normals for this area. Except for the above, considering agricultural, industrial and mining factors, we find that people reflect the productivity of their districts in their vitamin A test. That is to say, if the district is relatively high in productivity, the percentage normal will be relatively high and vice versa. Thus Brown is poor and Peace River, or Dark Brown is good. The differences are not large but are probably significant.

A relation was also found to exist between vitamin A and soil strips lying beneath the Parallels. Table XVI presents the data showing this relationship, and Figure IV is a map giving the same information.

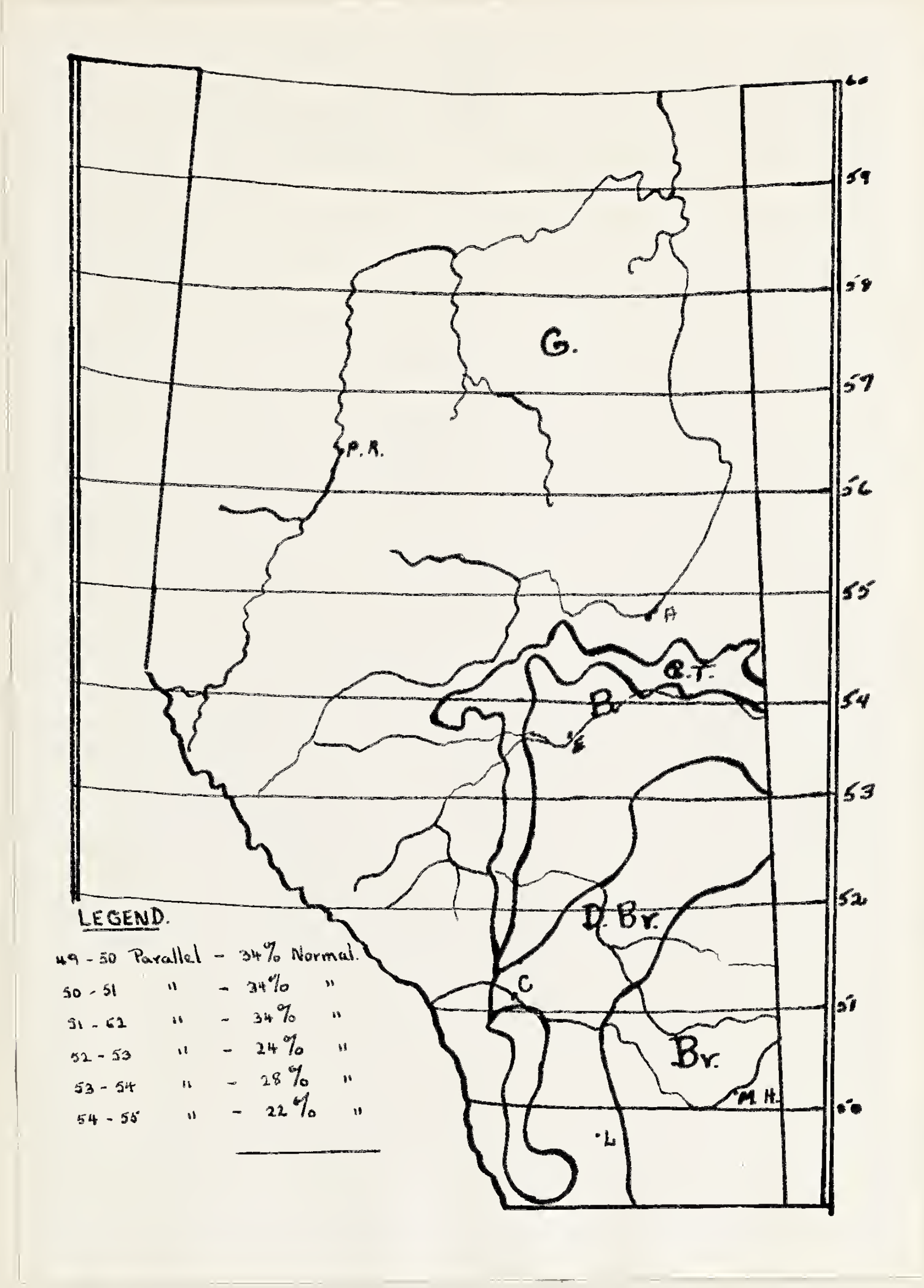

Figure IV

Relation of vitamin A deficiency to soil belts under the Parallels on a map of Alberta.

Table XVI

Percentage of Persons From Soil Belts Between Different Parallels Found To Be "Normal" in Vitamin A.

Parallels	Total No. of Persons	Percentage "Normal"
Below 50	128	34
50 - 51	90	34
51 - 52	112	34
52 - 53	218	24
53 - 54	268	28
54 - 55	157	22

Neither Calgary nor Edmonton are included in the above table.

On examining Table XVI and Figure IV, the results may be explained in the same way as those of Table XV. The percentage normal below the 50th Parallel, between 50-51, and 51-52 is the same, and relatively high. These sections(3) include approximately the same proportions of the 3 soil belts--Black, Brown and Dark Brown. The percentage normal drops above the 52nd Parallel, possibly along with the change in proportion of soil belts included in these sections. However, these differences do exist and their explanation is in doubt. They may be related to differences in standards of living for the various sections, productivity of the areas or industrialization, but we are not able to make any definite

statements until further work has been done.

2. Seasonal Variations

A group of about 175 students who had received vitamin A tests in October were retested in January. Table XVII presents the results of the cases studied.

Table XVII

Percentage of Persons Either Normal or Deficient in October Who Have Shortened Their Recovery Time, Remained the Same, or Lengthened Their Recovery Time

October Tests	January Retests			
	% Remained Same	% Became Shorter	% Became Longer	% Became Deficient
Normals 82	17	29	37	18
		Normal	Less Def.	More Def.
Deficients 94	6	42	29	22

We see that about 46 percent of the students who were normal in October remained about the same, while 37 percent became longer but still not deficient. However, 18 percent became deficient. Of the students who were deficient in vitamin A in October, 42 percent became normal by treatment or otherwise, 35 percent remained the same and 22 percent became more deficient.

Twenty-six percent of the students with shortened recovery times in January retests were known to receive treatment.

These figures show clearly a tendency for

adults to become more deficient as winter goes on, presumably reflecting changes in diet.

A similar survey conducted by Pett in the winter of 1938-1939 showed an even greater tendency to become deficient. However, these surveys will probably have to be conducted over several winters before conclusions can be drawn. The differences in the severities of the two winter seasons, 1938-1939 and 1939-1940, will no doubt explain to some extent the greater tendency towards deficiency in the first instance.

While conducting the retests to study seasonal variations, information on early symptoms of A deficiency was obtained from a questionnaire sent to 405 students. The results of the survey are summarized in Table XVIII.

Table XVIII

Percentage of the "Normal" Group and the "Deficient" Group Found to be Suffering From Various Conditions

Condition	Percentage Normals	Percentage Deficiencies
Consciously Night Blind	7	15
Eye Strain	17	27
Dry Nasal Mucosa	9	19
Dry Prickly Eyes	7	9
Dry Skin	6	12
Scanty Saliva	1	3
Colds (recent)	59	67

The only comment on the Table of results is that in almost all cases twice as many "deficients" are conscious of the various symptoms accompanying avitaminosis A as "normals".

3. The Effect of Feeding Vitamin A

Many cases found to be deficient in vitamin A received treatment or treated themselves with vitamin A from various sources. The results obtained from treatment of 226 persons with vitamin A showed a reduction in recovery time in all but 2 cases. In many cases under investigation the subjects themselves voluntarily reported evidence of improvement. In most cases, if doses of from 30,000 to 50,000 I.U. were taken daily for three to five days, the resulting recovery time was normal. However, if doses were smaller, the treatment was required over a longer period of time. In many cases it was found that the vitamin A requirement was exceptionally high or treatment period needed to be prolonged. This state is perhaps related to faulty absorption. In any case if subsequent treatment was followed, improvement in recovery time resulted.

~~In the case of A~~ One subject whose recovery time had been normal for some months, and who was not experiencing any of the symptoms of avitaminosis A, took a vitamin A-free diet for sixteen days. The tests and co-existent symptoms are given in Table XIX.

Table XIX

The Tests and Coexistent Symptoms of a Person (L.B.P.) On a Vitamin A Deficient Diet

Days On A-Free Diet	Recovery Time (Seconds)	Comment
1	6	Same recovery time for some time previous
2-4	7	
5-6	8	
7-8	9	Anorexia Stomatitis andDiarrhea Sore eyes ↓
9	10.2	
10	12	
11	13	Tires Easily ↓
12	14.6	
13	15	General Malaise ↓
14	16	
15-16	16	
16	14	8500 I.U. Vitamin A Taken
17	9	20,000 I.U. Vitamin A Taken
18	8	40,000 I.U Vitamin A Taken ↓
19-24	7	
25	6	Symptoms completely removed

The recovery time slowly lengthens as the supply of vitamin A in the liver is depleted. Moore, (1937), showed by analysis of the liver that when the supply of vitamin A to the body is stopped, the

reserves stored in the liver are slowly reduced, and consequently the supply in the blood will also be reduced.

Similar cases have been reported by Pett, (1939), Hecht and Mandelbaum, (1938), by Wald, Jeghers and Arminio, (1938). The subject reported by Pett, (1939), was the same as that cited above and similar symptoms and tests were reported. The experiment at that time was carried to a much more deficient state, but the prompt recovery resembled that of our case. The last two groups of experimenters, Hecht and Mandelbaum, (1938),and Wald, Jeghers and Arminio, (1938), studied similar problems, but the attendant symptoms were not completely considered.

4. Diurnal Rhythm

It was suspected that there is a daily rhythm for each person tested at regular intervals during the day. Table XX summarizes the results of testing 8 laboratory workers four times a day for five days.

Table XX

Recovery Times of Laboratory Workers Tested 4 Times a Day For Five Days

Subject	Days	Test in Early Morning	Test Before Lunch	Test After Lunch	Test Late Afternoon
(1)	1	8	10	13	7
	2	12	8	8	9
	3	9	4	4	6
	4	6	10	6	8
	5	12	11	12	9
(2)	1	21	17	19	19
	2	15	21	17	17
	3	20	20	17	17
(3)	1	7	6	5	6
	2	6	6	7	5
	3	7	7	5	8
	4	6	6	7	4
	5	8	6	6	6
(4)	1	9.6	11	10.6	8
	2	13	9	10	8
	3	10.6	10.6	11.8	12
	4	14	13.2	13.8	9
	5	16	16	12.2	12

Table XX (cont'd)

Subject	Days	Test in Early Morning	Test Before Lunch	Test After Lunch	Test Late Afternoon
(5)	1	14	11	9	8
	2	9	8	10	7
	3	9	8	10	7
	4	13	10.6	11	10
	5	10	9	8	8
(6)	1	6	7	7	5
	2	4	4	5	5
	3	6	3	5	4
(7)	1	9	9	10	8
	2	12	8	9	8
	3	12	9	7	8
	4	10	11	7	7
	5	14	9	9	9
(8)	1	10	10	10	10
	2	14	12	10	12
	3	9	13	9	13
	4	10	12	13	9
	5				
Average Test =		10.6	9.9	9.6	8.9

We may say from the above table, that the recovery time in the average test seems to decrease in length from early morning to late afternoon. Also, the tendency seems to be a shorter test in the late afternoon. However, although there is a slight rhythm shown, it is not significant enough to prevent tests being done at any time during the day, but it is of great physiological interest. The liver has a rhythm in releasing bile and sugar (Möllerström 1933), which can possibly be extended to the release and absorption of vitamin A as well. The results also show an inherent error of at least one second. There also may be a similar variation in the test for the same person from one day to the next. However, the accuracy of the test is only required within one second, so these results are within the limits of accuracy demanded.

Further work has been done on 10 persons who received a test every hour during the day for eight hours, starting in the morning and finishing in the late afternoon. The results of this experiment are summarized in Table XXI

Table XXI

Recovery Times Of Persons Tested Every Hour For Eight Hours During The Day

Subject	Recovery Times							
	9a.m.	10a.m.	11a.m.	12a.m.	2p.m.	3p.m.	4p.m.	5p.m.
1	20	24	34	24	29	29	25	23
2	8	12	12	11	9	9	7	6
3	13	16	23	18	20	20	14	14
4	11	13	20	19	15	15	16	12
5	7	7	10	14	8	12	15	11
6	8	13	23	12	12	13	17	11

Table XXI (cont'd)

Subject	Recovery Times							
	9a.m.	10a.m.	11a.m.	12a.m.	2p.m.	3p.m.	4p.m.	5p.m.
7	10	13	14	15	13	11	13	12
8	12	16	10	11	11	13	12	8
9	4	5	6	6	6	5	6	4
10	14	11	14	13	13	13	16	9

These observations are similar to those of the previous experiment. We may conclude that there are no special times during the day when tests should be done. Also, had tests been taken during the very early morning and late evening as well, the rhythm may have appeared more significant.

IV. BLOOD ANALYSIS.

Blood analysis is another method of testing for vitamin A deficiency. (Pett and LePage, 1940). Adaptation tests were done for LePage, and a good correlation between blood analyses and adaptation tests was found. Table XXII presents some of the results obtained.

Table XXII

Correlation Between Vitometer Readings (Recovery Times) And Blood Analyses

Recovery Times in Deficient Class.		Recovery Times in Normal Class	
Recovery Times	I.U. Vitamin A/100 ml Blood Plasma 1	Recovery Times	I.U. Vitamin A/100 ml Blood Plasma
28 sec.	46.6	10 sec	66.7
27 "	47.0	10 "	61.4
24 "	50.4	9 "	76.5
23 "	51.6	8 "	77.3
18 "	54.4	8 "	86.1
17 "	51	7 "	82.9
17.6"	51.2	7 "	81.9
16 "	55.5	7 "	80.5
15 "	55.7	6.25	84.8
14 "	64.1	6	87.8
13 "	56.2		
12 "	61.5		
12 "	68.0		
12 "	60.7		
12 "	73.4		

GENERAL SUMMARY AND CONCLUSIONS

The purpose of this investigation was to study thoroughly the many factors affecting the visual test for vitamin A deficiency and thereby to estimate the value of the Pett instrument for clinical use. The following results were obtained:

1. The question of wearing glasses during the test was found unimportant when 22 persons were tested with glasses on and retested with glasses off, after a 5 minute and ½ hour interval.

2. The effect of drugs on the test was observed when 6 persons who had been given a routine test received 2 drops of 2% pilocarpine nitrate in their eyes and were retested one hour and two hours after the administration of the drug.

3. The influence of light in the test room was found to be of some importance when 20 persons were given routine tests with 6 different light intensities in the room.

4. The effect of variation in bright light intensity (voltage) was studied on 20 persons.

5. Variation in the time of exposure to the bright light affects the test as shown in a study of 20 persons receiving tests at 6 different exposure times.

6. The danger that repeated tests on one individual might show an error due to "practice" or "learning" was studied in two ways: (a) seventeen persons were each given 15 tests at one minute

intervals, with only one person showing a learning curve. (b) Twenty-five persons were each given a single test(not the median of three tests), on each of 21 successive days, with eleven of the twenty-five showing learning. In both experiments the "learning" effect could be abolished either by lengthening the interval between the tests or by following the prescribed method of doing three tests and using the median.

7. 1600 people from different parts of Alberta were charted on a map of the Province and the result was found to relate deficiency in vitamin A to soil belts.

8. A tendency of adults to reflect seasonal changes in diets was shown in a survey of 175 students, tested at intervals throughout the winter.

9. This test for vitamin A deficiency was also correlated with symptoms of avitaminosis A through a survey of 405 Freshmen.

10. The effect of feeding vitamin A to a person who had become deficient due to an A-free diet and the effect on persons deficient and normal were studied.

11. Evidence of a change in vitamin A level during the day is shown in a study of 20 persons tested every hour for 8 hours during the day.

12. Blood analysis is another method for testing for vitamin A deficiency. Assistance was given in

a study which showed a correlation between blood analysis and this adaptation test.

As a result of this investigation, we may conclude that under the conditions stated for the operation of the Pett tester for vitamin A deficiency:

1. Glasses, if worn by the individual, may be kept on for the test for the sake of speed. There is no effect on the test if glasses are removed.
2. Drugs in the eyes which cause the pupil either to contract or to expand, affect the test (unless adequate time is allowed for adaptation to the drug.)
3. The test room is best kept relatively dark during the test, but small amounts of light may be allowed in, provided the light be kept constant for all tests on the same person. The optimum amount of light allowable in the test room varies somewhat with the individual.
4. The time of exposure to bright light (30 seconds in the Pett procedure) may be fixed at any time between 25 and 40 seconds.
5. The bright light, maintained at a fixed intensity in the Pett procedure of 50 foot-candles (or about 100 volts), may be fixed

at any intensity between 85 and 100 volts and give consistent results.

6. There is a disturbing learning factor to contend with in repeated tests only in rare cases. This is important clinically in following the effects of treatment.
7. There is some evidence that persons living in certain soil belts of Alberta are more deficient in vitamin A than persons living in other soil belts. (There is, therefore, a correlation between vitamin A deficiency and the soil belts.)
8. There is a tendency for adults to reflect seasonal changes in diets in their vitamin A test, becoming more deficient in winter than in summer.
9. A person on an A-free diet for some time exhibits vitamin A deficiency as shown in a prolonged recovery time when tested by the Pett machine, as well as pathological symptoms. Also, deficient cases can be cured or brought to "normal" recovery time in 3 to 5 days with vitamin A therapy.
10. The test may be taken any time during the day.

APPENDIX

Table IV

Recovery Times of 20 Persons Tested With Six Different Light Intensities in the Test Room

Subject	Intensities At The Headpiece of The Machine in Foot Candles					
	21/2 and 1/2	11/2 and 3	4 and 4	5 and 5	6 and 6	regular test
(1)	7	7	7	6	5	4
(2)	9	9	12	9	11	10
(3)	10	12	12	11	10	10
(4)	26	23	24	27	25	20
(5)	15	24	29	16	15	19
(6)	15	13	17	10	19	14
(7)	13	13	10	15	7	7
(8)	10	7	8	11	10	8
(9)	6	11	11	10	11	7
(10)	16	13	20	15	14	15
(11)	7	10	10	8	9	10
(12)	9	9	9	9	14	12
(13)	11	12	10	9	7	8
(14)	15	8	14	11	9	8
(15)	17	22	21	22	21	24
(16)	8	8	8	7	8	6
(17)	17	13	12	14	18	15
(18)	12	12	12	13	12	12
(19)	12	10	11	13	14	10
(20)	10	12	9	9	9	12

Optimum range of light intensities at the headpiece of the Pett Machine varies with the individual

VI

Table V

Recovery Times of 20 Persons Tested With The Bright Light At Six Different Intensities -- Brought About By Changing Voltage

Subject	Recovery Times At Different Voltages					
	80 volts	85 volts	90 volts	95 volts	100 volts	105 volts
(1)	10	9.6	10.2	7.8	7.6	9.4
(2)	8	7	9	7.6	8	8.8
(3)	8	9	9.6	10	10	10
(4)	6	6	7	7	7	7
(5)	5	5	5	5	5	7
(6)	4	5	4	2	3	4
(7)	9	8	8.8	8.2	9	11
(8)	6	7	7	6	7.2	8
(9)	13	9	11	13	13	17
(10)	7	8	7	8	11	13
(11)	5	7	6	6	14	9
(12)	9	11	11	12	15	21
(13)	13	14	11	14	16	21
(14)	7	6	11	17	14	14
(15)	21	20	19	8	19	18
(16)	5	5	7	11	9	10
(17)	11	10	13	9	13	15
(18)	9	8	9	8	9	9
(19)	26	24	18	22	20	24
(20)	12	9	13	12	11	13

The optimum voltage range is from 85 to 100 volts

Table VI

Recovery Times of 20 Persons Tested In Triplicate At Various Different Exposure Times To The Bright Light.

Subject	Tests at Various Times in Seconds											
	5	8	10	12	15	18	20	25	30	40	50	60
(1)	6.6	7.4	10.0	9.0	12.0	13.0	11.0	15.0	16.0	20.0	16.0	18.0
(2)	4.0	3.0	4.0	3.3	4.0	4.0	4.5	4.6	6.4	6.2	7.0	7.0
(3)	5.0		5.0		6.1		8.6	7.8	6.6	7.0		9.8
(4)	4.0		4.6		4.0		4.6	4.4	6.4			6.8
(5)	5.0	5.2	6.4	7.0	6.8		7.0	10.0	10.2	9.0	10.0	13.4
(6)	5.0	3.8	4.8	4.0	5.4		6.2	7.2	7.4	7.4	7.2	10.6
(7)	4.0	3.4	4.8		5.2		5.6	6.0	6.8	7.8	8.2	10.2
(8)	5.0		4.4		6.4		6.6	6.8	10.2			12.8
(9)	5.0	6.4	6.0	6.0	7.8	4.8	6.0	6.0	9.8	13.0	11.0	11.0
(10)	4.0	3.4	3.8	3.8	4.2		5.0		7.2	6.8	8.4	7.2
(11)	4.0	3.8	4.6	4.8	4.8	5.6	6.4	6.6	6.6	8.4	9.8	9.8
(12)	5.0	4.8	5.2	5.2	5.6		6.2	6.8	6.8	11.0	12.0	16.2
(13)	11.0	9.0	7.8	11.6	13.8	11.8	16.2	14.2	17.4	17.8	20.6	18.2
(14)	5.0	5.0	8.4	8.0	11.8	11.8	12.4	12.4	11.4	14.6	14.2	20.0
(15)	6.0	5.4	5.0	5.2	5.2	6.2	6.0	7.8	8.6	9.0	11.2	12.0
(16)	3.0	4.8	4.6	5.6	6.0		7.4	9.2	8.0	8.4	9.4	11.2
(17)	4.0	4.4	5.2	5.8	7.6		8.4	8.6	10.2	10.2	12.2	11.4
(18)	5.0	6.4	6.4	7.0	9.2		9.8	15.0	12.0	15.0	15.0	15.6
(19)	4.0	4.0	6.4	6.6	7.2		6.8	10.0	11.0	13.0	14.0	14.0
(20)	5.0	6.0	8.2	7.4	7.4		7.4	12.4	8.8	11.8	14.4	25.0

The optimum range in exposure times is from 25 to 40 seconds or possibly 20 to 40 seconds.

Table VII

Recovery Times of 17 Different Persons Given Single Tests At Various Different Exposure Times To The Bright Light

Subject	Tests At Various Exposure Times In Seconds											
	5	8	10	12	15	18	20	25	30	40	50	60
(1)	4	10	7.4	8	10	9.4	9.2	12.8	12.6	19.2	20.8	18.8
(2)	5	3	5	4	4	4	5	6	6	6	10	6
(3)	2.6	4.8	5.4	6.6	7		6.8	8	11.2	11.4	11.2	13.6
(4)	3.4	3.4	3.4	4	4.6	4.2	4.8	4.4	5.2			6.2
(5)	4.2	5	6	6.2	7.6	7.6	9	9.6	9.2	9.8	9.8	9.8
(6)	5	5	3	4.8	5.8	6	5.8	5.8	7	8.8	9.2	10
(7)	3.2	4	4.8	3.2	5		4.2	5.2	5.8	7.4	9.8	10
(8)	3.2	5.6	4.4	5	5.8	5.8	5.8	5.6	7	8.4	8.6	13.8
(9)	5.2	5.2	5		6	5.2	5.8	6.8	7.2	7.4	7.4	12.6
(10)	5.2	4.2	4	4.2	4.4	5.8	6.2	6.2	6.2	6.2	6.8	6.8
(11)	4.4	4	4.2	4.8	5.8	6	6.8	7.2	7.4	8.4	12.2	9.4
(12)	5.4	10	7.8	9.8	11.6	10.6	13.6	12.5	12.2	13	11	15.6
(13)	5.2	6.2	7.4	8.2	13.6	13.2	15.2	14.2	16.8	21.2	21.6	23
(14)	5	6.8	6	7.6	8	8.6	6.4	11	11.6	14.6	16.4	12.8
(15)	4.6	4.8	6.6	4.8	5.4	5.2	5.8	5.2	7.2	9.8		10
(16)	3.8	5.6	6.6	6.4	6.4		9	8	7.4	10.6	11.4	15.6
(17)	4	10.2	8.2	8	8.8		12	12.6	9.8	13.4	16.4	21.6

The optimum range in exposure times when single tests are taken is also between 25 and 40 seconds, and possibly 20 and 40 seconds.

Table VIII

Recovery Times of 17 Persons Given 15 Consecutive Tests With Only One Minute Intervals Between Tests.

Sub-ject			Recovery Times												
(1)	6.4	7.8	4.8	5.2	3.8	4.6	5.0	3.2	3.8	4.0	5.4	4.0	5.2	3.4	3.8
(2)	22.8	23.8	21.0	14.8	10.8	13.2	13.2	8.8	8.8	9.0	13.8	10.4	12.0	9.2	7.0
(3)	5.6	5.6	4.6	5.0	3.8	4.2	4.8	4.6	4.8	5.0	4.8	3.6	4.6	4.4	4.8
(4)	7.0	9.0	7.0	11.0	10.0	9.0	10.0	9.0	10.0	7.0	9.0	8.0	9.0	11.0	8.0
(5)	15.0	18.0	13.0	16.0	14.0	12.0	16.0	13.0	18.0	14.0	13.0	13.0	22.0	14.0	17.0
(6)	10	23	18	18	7	23	14	16	17	13	14	16	12	13	
(7)	24	13	10	10	15	13	14	12	14	14	9	13	14	11	13
(8)	11	7	10	11	5	6	7	6	10	10	6	6	8	7	5
(9)	20	18	12	10	15	19	18	20	14	14	17	15	21	17	10
(10)	19	10	11	10	14	12	13	11	12	14	12	20	14	21	14
(11)	12	12	14	10	11	10	12	13	21	14	9	15	19	15	9
(12)	10	19	11	10	10	12	13	12	10	10	11	13	9	8	9
(13)	16	18	19	18	14	17	17	16	16	11	21	20	13	15	12

Table VIII (cont'd)

Subject	Recovery Times														
(14)	11	12	10	9	15	12	11	13	11	11	15	12	9	10	8
(15)	15	14	14	17	11	13	9	16	12	11	9	13	15	11	15
(16)	10	19	10	9	10	14	15	16	17	9	10	10	8	10	9
(17)	23	16	24	18	25	18	20	22	16	21	15	17	21	12	20

Only one person, subject (2), showed any tendency towards learning with repeated tests.

Table IX

Recovery Times of 3 Persons (Subjects (1), (2) and (3)) Given Fifteen Consecutive Tests With Five Minute Interval Between Tests.

Subject	Recovery Times														
(1)	2.4	4.6	3.6	3.8	3.8	3.8	3.6	5.0	4.6	4.8	5.0	3.6	3.4	4.0	3.6
(2)	11.4	14.8	15.0	9.0	9.8	13.4	8.2	14.2	13.6	12.8	13.2	13.0	9.0	12.8	12.0
(3)	4.4	4.2	4.2	3.8	4.0	3.0	3.6	4.0	4.0	3.8	3.6	3.6	3.6	3.4	

No learning is shown when the interval between tests is increased from one to five minutes.

Table X

Recovery Times of 25 Persons Given One Test a Day For Twenty-one Days

Sub-ject			Recovery Times																		
(1)	22	17	21	15	18	14	14	14	13	14	11	16	12	17	14	12	14	14	19	14	20
(2)	20	17	19	19	18	14	13	12	15	15	17	14	12	9	13	8	10	14	11	8	9
(3)	12	12	22	18	11	8	8	10	7	6	8	8	8	6	8	6	6	6	10	8	7
(4)	12	15	14	16	10	10	10	11	11	10	9	13	12	11	11	12	11	14	14	12	16
(5)	14	20	20	17	11	24	16	19	14	18	25	14	26	16	18	23	18	29	24	25	46
(6)	18	22	18	22	18	17	21	15	11	13	12	19	14	13	12	13	10	10	11	14	16
(7)	20	21	18	15	13	7	17	12	25	15	18	11	11	15	10	15	11	30	20	21	17
(8)	26	26	20	39	18	22	18	33	16	27	11	9	9	10	13	13	13	11	15	15	18
(9)	19	13	10	9	17	16	10	16	10	9	8	8	9	9	12	8	8	8	8	9	11
(10)	17	15	12	17	13	12	15	15	15	10	14	15	14	13	12	9	12	13	11	10	13
(11)	29	18	17	17	13	10	12	10	13	10	13	8	12	12	12	12	8	15	17	17	18
(12)	27	20	20	13	10	14	16	16	10	16	16	14	15	14	11	15	10	13	13	14	14
(13)	13	9	6	10	6	9	9	8	10	12	7	7	9	6	8	5	7	8	6	6	7
(14)	7	7	7	7	4	5	5	5	5	6	4	6	6	6	6	5	6	4	4	6	4

Table X (cont'd)

Sub-ject	Recovery Times																				
(15)	15	10	12	11	17	16	12	12	13	16	13	11	12	12	13	12	13	14	10	16	13
(16)	26	28	34	14	8	14	16	18	16	17	11	14	9	9	9	9	8	8	8	9	8
(17)	7	7	4	6	13	9	8	7	4	5	4	4	7	-	4	4	4	6	3	4	4
(18)	18	10	8	8	6	8	6	6	7	4	5	7	7	6	5	7	6	5	6	5	6
(19)	16	18	15	15	26	15	16	14	16	14	12	20	14	18	14	15	16	12	13	11	12
(20)	22	6	8	6	6	4	6	6	5	6	6	5	4	5	4	4	8	4	4	4	
(21)	17	15	11	15	18	8	10	17	20	16	19	13	12	14	12	16	12	12	13	13	
(22)	10	8	6	8	9	8	5	5	7	6	6	6	5	-	5	6	7	7	7	6	8
(23)	16	12	12	20	17	16	8	13	11	16	14	14	9	12	17	8	15	14	9		
(24)	5	4	4	5	6	3	3	10	9	4	9	8	6	9	5	5	4	5	4		
(25)	22	28	17	14	19	19	29	36	30	24	23	26	17	13	27	19	20	29			

Learning with repeated daily tests is shown in 11 cases, (2), (3), (6), (8), (9), (11), (12), (15), (16), (18), (20).

Table XI

Recovery Times of 10 Persons Given Three Three Tests A Day
(The Median of the Three Tests is Used As the Recovery Time)--
For Several Consecutive Days

Subject	Recovery Times										
(1)	7	7	7	7	6	7	7	7			
(2)	15	13	12	15	13	14	12	10	10	13	
(3)	11	11	9	10	11	10	10	10	10	10	
(4)	12	14	11	12	13	14	12	12	15	12	
(5)	4	4	4	5	4	6	6	4			
(6)	20	10	20	18	17	15	13	19	19		
(7)	20	24	21	20	22	22	21	22	24	17	21
(8)	14	13	13	24	17	14	13	31	20	13	15
(9)	13	12	14	12	19	17	16	17	17	18	
(10)	13	14	12	18	16	14	13	16	11	14	15

No learning with repeated tests is shown, if the median of three tests be taken as the recovery time.

Mathematical treatment of results presented in Tables VII and X.

In calculating σM for Table X we found them to be:

± (3.1, 4.3, 4.2, 2.2, 7.4, 3.7, 5.2, 7.9, 3.3, 2.1, 5.8, 3.7, 2.0, 3.4, 2.0, 7.3, 1.9, 2.4, 1.4, 3.9, 1.5, 1.0, 3.5, 2.9, and 5.4) with the mean deviation = ±3.6.

σMe was found to be ± (7.3, 5.0, 6.2, 4.8, 11.3, 7.0, 8.3, 11.7, 6.8, 3.1, 6.8, 5.9, 2.0, 11.2, 3.4, 4.8, 1.8, 5.0, 1.9, 2.0, 5.0, 2.9, 10.4) and the mean deviation = ±5.6.

σ_M for Table VIII were ±(.9, 5.1, 0.0, 1.3, 2.6, 4.2, 3.2, 1.9, 3.4, 2.7) with a mean deviation of ±2.5.

σ_{Me} = ±(2.5, 9.0, 0.9, 0.7, 6.5, 5.2, 4.2, 3.8, 6.5, 5.5) and the mean deviation here - ±4.5.

σ_M was also calculated on every twentieth person out of 820 persons who were given the prescribed three tests, and the results were: ±(1.5, 2.2, 0, 0.8, 1.6, 3.6,1.6, 3.2, 2.9, 1.5, 0.1, 2.9, 1.6, 2.8, 2.4, 7.1, 5.4, 2.8, 0, 7.7, 0.8, 0, 1.4, 2.2, 1.7, 2.4, 0, 1.7, 2.1, 0.8, 0.8, 6.3, 3.9, 7.2, 6.2, 3.2, 7.3, 0, 4.1) with a mean deviation of ±2.5.

The σ_{Me} were ±(3.7, 3.8, 0, 2.7, 3.5, 8.3, 3.1, 7.5, 5.9, 3.0, 4.5, 7.7, 3.8, 10.4, 5.6, 13.4, 8.3, 7.0, 0, 15.4, 0.8, 0, 4.8, 4.0, 1.7, 2.4, 0, 0.8, 6.3, 0.8, 0.8, 0.8, 6.3, 10, 11.8, 4.7, 5.5, 11.9, 0, 4.1) with a mean deviation of ±4.7.

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